

# Brushing Your Spacecraft's Teeth: A Review of Biological Reduction Processes for Planetary Protection Missions

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*Abstract*— Much like keeping your teeth clean, where you brush away biofilms that your dentist calls “plaque,” there are various methods to clean spaceflight hardware of biological contamination, known as biological reduction processes. Different approaches clean your hardware’s “teeth” in different ways and with different levels of effectiveness. We know that brushing at home with a simple toothbrush is convenient and has a different level of impact vs. getting your teeth cleaned at the dentist. In the same way, there are some approaches to biological reduction that may require simple tools or more complex implementation approaches (think about sonicating or just soaking your dentures, vs. brushing them). There are also some that are more effective for different degrees of cleanliness and still some that have materials compatibility concerns. In this article, we review known and NASA-certified approaches for biological reduction, pointing out materials compatibility concerns and areas where additional research is needed.

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## 1. INTRODUCTION: WHY BRUSH YOUR SPACECRAFT’S TEETH?

Why do it? Why have that routine of brushing before bed and when you wake up? The simple answer: microbes grow. They’re not like dust particles or non-living particles of any kind. The organisms in your mouth live and grow and thrive on what’s left behind when you eat. At the end of the day (or night), if you don’t kill them, you have more microbes than you started with.

Granted, your spacecraft hardware isn’t inhabiting the same warm and moist environment as your mouth. It’s likely in at least an ISO 8 cleanroom, if not cleaner. That may give you some comfort, though the ISO standards speak only to the particle content, not the temperature or relative humidity [1]. In general, most spacecraft cleanrooms exist at about 70°F/20°C and about 50% humidity. While it’s not the environment in your mouth, as anyone who has a kitchen counter can witness, things are still able to grow in that climate, especially if there is plenty of growth medium (remember that loaf of bread from two weeks ago?). While there are hopefully no loaves of bread in your cleanroom, there is usually enough starting material to sustain microbes at that temperature and humidity.

NASA’s planetary protection requirements focus on the hardier of the microorganisms that can exist, known as spores [2]. Spores are a dormant form of living microbes. Wrapped in their protective coats, they lie in wait for the right time, temperature and humidity to emerge from their silent hibernation and start to grow. Some bacteria, under the right conditions, can double their numbers every 17 minutes. In contrast, when looking at the power of a given bioburden reduction technique on a spore, we refer to how many *log-reductions* of spores take place (the change from the starting to ending population of spores in orders of magnitude in powers of ten). Unless specifically identified, this paper will focus on bioburden reduction of spores and spore-forming

organisms, rather than non-spore formers or their vegetative friends.

As our search for life expands from Mars into the Ocean Worlds, it is important to review the diversity of available techniques to reduce bioburden on spacecraft hardware to insure compliance with planetary protection requirements.

Broadly speaking, bioburden reduction techniques address different levels of hardware, depending on the degree of penetration of the technique. Some approaches address only the bioburden existing on the *surface* of a material, while other techniques that *penetrate* address the more interior portions of the hardware—in either the interior of a nested structure, a porous/diffusive structure or a structure that has been integrated<sup>1</sup>.

## 2. BRUSH AND SWISH: SURFACE BIOBURDEN REDUCTION TECHNIQUES

Surface reduction techniques for bioburden fall into the same camp as brushing your teeth—mechanically and/or chemically interacting with a given surface for the purposes of physically removing and/or chemically interacting with the microbial inhabitants of the surface... a brush and a swish.

### *Physical Removal Techniques*

*Solvents* Ease of access often make solvents the first line of bioburden reduction for hardware builders to turn to. Solvents work by chemically reacting and/or dissolving surface inhabitants. When paired with a physical method, such as wipes or ultrasonic application, there is an additional physical removal of material. Solvents may be applied by wiping, ultrasonic baths or other means that have an additional capacity to physically remove bioburden as well as chemically inactivate it. Solvent efficacy is influenced by geometry and surface energy of the solvent relative to the material surface properties of the surface of interest and microbial adhesion energy. The application of a single solvent to a diversity of materials—metals and non-metals doesn't always lead to the same log reduction on a given surface. As it is with many things in life, one size does not fit all.

The majority of common solvents used on spaceflight hardware do not have the ability to kill or biologically reduce spores (*sporicidal*). For example, isopropyl alcohol does not kill spores. The same can be said of acetone, methanol, ethanol and acids—they are *sporeostatic*—they only inhibit germination and any additional outgrowth of spores, but the spores are still alive and well. These commonly used solvents may, in combination with ultrasound or mechanical force from wiping, assist in the physical removal of spores by mechanical removal of adhered spores, but it does not act as

a sporicide. Common sporicidal solvents include: glutaraldehyde, iodine compounds, chlorine compounds, peroxyacids, hydrogen peroxide, ethylene oxide and 13-propiolactone [3].

*Foams* Akin to solvents, foams have been developed by the Department of Defense/Sandia for the purposes of decontamination after an Anthrax scare. Challenge spores commonly used in testing these foams are comparable to those used by NASA (*G. stearothermophilus*). Foams were invented as an alternate to solvents, due to the physical nature of a foam, which would allow penetration into various geometries and surface finishes, including porous media on the size scale of an individual foam bubble or larger [4].

Like solvents, foams are influenced by the starting organic load [5]. At this time, materials compatibility studies are limited and a neutralizer is often applied after application of the surface decontamination foam in order to halt any reaction between the foam and material under reduction. A 4-log reduction can result if the foam is applied to a surface for 24 hours, setting a boundary on materials compatibility with foams. Scalability appears to be a straightforward operation, though multiple interfaces and joints may not be readily accessible. Resistance is unknown.

*Carbon Dioxide* Carbon Dioxide has a phase diagram that permits a supercritical fluid state. As a solid, CO<sub>2</sub> can be delivered to a surface as a jet that mechanically removes micron-sized particulate, akin to sand- or bead-blasting, known as CO<sub>2</sub> “snow” [6]. CO is often employed in the food and medical industry when heat- and chemical-sensitivity are of concern. There is a small elevation of temperature between 30-50°C and pressures between 10-100 atmospheres, though typically that range of temperatures is not a threat to most surfaces. This approach has been shown to be effective for the removal of micron-scale particulate contamination, though it is not effective for spore inactivation or removal, unless paired with other active modifier solutions [7]. The majority of papers on the use of supercritical CO<sub>2</sub> and CO<sub>2</sub> snow that actually show bioburden reduction of spores are often combined methods (e.g. CO<sub>2</sub> + peracetic acid or reduction by sterile filtration with the use of CO<sub>2</sub> as the filtration solvent). A comprehensive review of supercritical CO<sub>2</sub> for sterilization can be found here [8]. This approach may be challenging to scale up to a subsystem or system level due to the delivery method of the CO<sub>2</sub>, which is limited to a small spot size. There is no NASA or ESA approved process for using supercritical CO<sub>2</sub>.

### *Radiation-Based Techniques*

*Ultraviolet* The medical industry commonly employs the use of Ultraviolet (UV) radiation. At around 254 nanometers (nm), UV radiation has the energy to break microbial DNA, rendering it unable to reproduce or grow and in some cases,

<sup>1</sup> Note that the topics of embedded bioburden & mated surfaces will be discussed in a future paper.

altogether perish. UV radiation in the range of 45-80 milliJoules/cm<sup>2</sup> between 254-263 nm typically affects *B. Subtilis* spores. The unfortunate features of UV radiation are that geometry, shadowing, distance and the initial level of surface contamination influence available intensity, further driving down its sterilization potential. [9]. At present, neither a NASA nor ESA certified process for the use of UV exists.

Ultraviolet can only reach surfaces with direct exposure. It cannot be used with interiors, shadowed surfaces or holes. The penetration depth of the sporicidal wavelengths of UV light is so short that even a layer of spores is sufficient for protecting a layer of spores beneath it from harm [10].

The sun is abundant and there is an obvious temptation to employ UV for post-launch bioburden reduction. That particular enticement may arise when a project may want to pursue alternate destinations than were intended for the mission, as the science may be so compelling as to drive a desire to voyage to areas that hardware may not have received or a mission may have cost-schedule-infrastructure constraints that drive a planetary protection philosophy that places the onus on post-launch activities.

Declaring the use of UV for flight hardware sterilization is not a *fait accompli* for bioburden reduction, as nature seems to always find a way to show clever ways to adapt. There is increasing evidence that successive generations of UV irradiated spores develop a resistance to UV [11], [12], [13]. In low water content environments, there may be an additional level of protection imparted to spores, as resistance has been observed in low-water content *B. Subtilis* spores [14].

The fact that a spore may develop resistance and the ability to develop it is neither uniform, nor well-characterized makes it difficult, if not impossible to take a process-based approach to using UV for bioburden reduction without verification. Applying a certain time, fluence and wavelength range may not be sufficient—there may be one (or several) of the strong that survive and live to procreate another day.

As an additional mention to those building life-detection hardware, if UV does completely kill a spore, the spore is not removed by the UV source. It remains on the surface, with the spore core leaving a potentially substantial signal of dipicolinic acid, which exists in a spore core and may confound life-detection measurements [15].

*Infrared* Infrared (IR) radiation has had limited investigation for use as a bioburden reduction process. Infrared works by local thermal degradation of the spore coat and internal spore contents. It has been shown that *B. subtilis* (ATCC 9322) is reduced by 6 logs in under 10 minutes when exposed to IR, though other references show promoted germination of *B. subtilis* when exposed to IR [16]. Overall, there is limited work in the literature on IR process parameter dependence,

variation in spore responses and adaptation. Scalability may be an option due to the availability of large-scale IR sources.

#### *Techniques Employing Reactive Chemical Species*

There are several bioburden reduction approaches that involve the generation of one or more reactive species. These reactive species are typically able to oxidize or otherwise react with a spore coat, disrupt it and enter into the core of a spore, destroying it. Plasma, ethylene trioxide, nitrogen dioxide, ozone and hydrogen peroxide are the most common forms of reactive chemical species employed for bioburden reduction.

Reactive chemical species techniques are often employed in the bioburden reduction of materials that are intolerant to high-temperatures and humidity. Some awareness of corrosion susceptibility and etch rates needed when exposing polymers to any of these techniques to insure that material loss is not a concern.

*Plasma* Plasma is a partially ionized gas that is composed of ions, radicals, electrons and uncharged species (atoms and molecules). Plasma kills spores primarily by the charged species--reactive oxidative species and charged particles, which disrupt the spore coat. While there is some UV present in plasma, several observations show that there is no measurable UV output for wavelengths less than 290 nm. Plasma can be either thermal or non-thermal ("cold") depending on the thermalization of electrons. [17]

Some cold plasma-based approaches show 3-4 log reduction of *Deinococcus Radiodurans* at room temperature at atmospheric pressure [18] and 4-6 log reduction for *G. stearothermophilus*, *B. subtilis*, *B. atrophaeus*, and *B. pumilis* [19]. Oxygen plays a role in reducing some species, such as *B. subtilis*, but not others [20], [21]. There are no studies at present on resistance to plasma-based reduction approaches. In addition, scalability is currently limited by the ability to raster a cold plasma jet across a large surface. Cost is a third consideration here, though the benefits associated with the increased log reduction are worthy of further investigation. There is no NASA or ESA standard process for the use of plasma at this time.

*Ethylene Trioxide* Though well-studied during the 1960s and 1970s at NASA, ethylene trioxide has fallen out of use, as it has often been paired with a chlorofluorocarbon (CFC) sterilizing agent, which were phased out with the Clean Air Act in 1995. Even with alternate stabilizers or no stabilizer at all, ethylene trioxide requires a state-enforced an environmental abatement program that makes its use cost-ineffective [22].

*Nitrogen Dioxide* Nitrogen dioxide gas can be generated at room temperature by a few different approaches. Absorbed NO<sub>2</sub> degrades DNA in the spore core due to its reactivity. Hardware needed. Cycle times are on the order of minutes for 6-8 logs reduction of *G. stearothermophilus* and *B. Subtilis*.

This approach is still in its infancy, so materials compatibility and resistance information are quite limited [32].

*Ozone* Ozone is a reactive species of oxygen—O<sub>2</sub> with a loosely bonded third oxygen. This reactive species is often formed by the acceleration of oxygen at high voltages. Ozone kills spores by degrading their outer coat, exposing the inner core to the reactive oxygen species [23]. Commercial systems are available, though not scaled to a size that would permit larger than part-level sterilization. This approach may be suitable for tool sterilization for aseptic assembly, as the process duration is short and a tabletop ozone system is inexpensive. There is no NASA or ESA standard process for the use of ozone, though this should not limit flight projects from proposing a process for use on tools during aseptic assembly, which should be straightforward.

*Hydrogen Peroxide* When delivered as a vapor, hydrogen peroxide has been found to be an effective bioburden reducer. Vapor Hydrogen Peroxide (VHP) is generated when liquid hydrogen peroxide is either thermally vaporized or pulled by pressure using a carrier gas into a vacuum chamber. Commercial systems are readily available for the delivery of vapor phase hydrogen peroxide. VHP has good materials compatibility (both metals and non-metals) and operates near room temperature. For example, the 2024 and 7075 series for Aluminum, 304 stainless steel show no changes in mechanical properties after VHP treatment. Composite systems such as Carbon Fiber/Epoxy (CF/E) and Carbon Fiber/Glass Fiber-Epoxy (CF/GF-E), or uncoated FR4 show no change in chemical or mechanical properties [24]. European Space Agency has developed a validated process for the use of VHP in planetary protection missions which NASA has accepted, so process parameters are immediately available for use [25].

### Making Decisions

Geometric awareness is critical with the use of surface techniques. Corners, crevices and blind ends are not easily accessible and must be considered, lest a false sense of security wash over an implementer.

A subset of geometric awareness is knowing the relative difference between the roughness of the surface to be cleaned and the roughness of the tool under use. This gives rise to an efficiency factor for the removal of bioburden. The first paper in this series, in 2016, looked at the effect of surface finish and materials composition on the relative habitability of different materials. The use of wipes, swabs or other surface-contacting media that are coarser than the surface roughness of a given material may lead to incomplete removal of bioburden and a false sense of bioburden reduction security.

### Some Pro Tips

Without appropriate pre-cleaning, surface bioburden reduction processes are not as effective as one may believe. The presence of organic and inorganic matter may shadow, mask or support bioburden present on a surface. In addition,

organic material can influence the available reactivity of a solvent or solvent physically available for dissolution of bioburden. So, the assumption here is that basic cleaning of surfaces has occurred to remove particulate and organic soils.

In addition, it makes little sense from a cleanliness perspective to recycle solvents or gases or to use ovens and other hardware that has not been cleaned and handled with the utmost care and concern for recontamination to the hardware undergoing reduction. Simply put: make sure that your toothbrush has been well-rinsed and not sitting on the floor before you use it [26]!

Finally, it's worth noting that the effects of applying multiple bioburden reduction techniques are not readily additive. For example—the use of two different one-log reduction techniques doesn't necessarily equal a two log reduction. Those two different one-log reduction techniques may influence different organisms that have been tested, known as *challenge organisms*, which represent the hardiest of the lot that have been found thus far for this particular approach. It may be that one approach reduces the NASA Standard *B. Subtilis* by 1 log and another approach reduces *B. pumilis* by 1 log, this doesn't mean that you've ended up with a 2-log reduction process.

### Summary

Surface bioburden techniques are often conducted at or near room-temperature. They have varying degrees of chemical reactivity and varying degrees of bioburden reduction, as summarized in Table 1. The majority of surface bioburden techniques leave residual dead bodies, which may interfere with signal detection, unless combined with a method that mechanically removes microbes from the surface.

**Table 1. Summary of Major Surface Bioburden Reduction Techniques**

Technique	Log Reduction Range	Possible Spore Resistance?	Residual Dead Bodies
Solvent	NA	Possible	Partially
Foam	4	Unknown	Partially
Ultraviolet	< 2	@low water activity	Yes
Infrared	2-6	Unknown	Yes
Super CO <sub>2</sub>	< 1/None	NA	Partially
NO <sub>2</sub>	4-8	Unknown	Yes
Plasma	2-4	Unknown	Yes
ETO	4	Yes	Partial-none
VHP	4-6	Yes	Partial-none

### 3. DEEP CLEANING: PENETRATING BIOBURDEN REDUCTION TECHNIQUES

#### Background

**DHMR** Dry Heat Microbial Reduction (DHMR) is likely the first approach that most aspiring planetary protectors are exposed to when evaluating sterilization techniques for missions requiring bioburden control. While the name does state that “dry heat” is used to microbially reduce bioloads on hardware, it is in fact the case that a small amount of humidity is introduced into the system on a controlled basis. Dry Heat Microbial Reduction specifically targets. The NASA standard requirements have expanded to a broader range of time, temperature and humidity: D-values have been developed for T = 125-200°C to account for the reduction of hardier microbes. Since the 1960s, NASA has invested in the qualification of hardware that is DHMR-friendly. There are overlaps with high temperature component-level specifications for parts tested in the high-temperature limit under MIL-SPEC 810F. Both NASA and ESA have approved processes for DHMR [27].

This paper is being written in the month before Thanksgiving, where thoughts turn towards the simplicity of DHMR in daily life: the cooking of the Thanksgiving turkey. Most of you will have popped the bird in the oven (or Tofurky) at 350-425°F for 4 - 6 hours. The time and temperature are set by the USDA guidelines for the number of microbes reduced over a period of time. The Celsius equivalent is 177-218°C, for 4-6 hours. As a comparison, the DHMR time and temperature equivalent at 110°C (lower temperature) is 50 hours, though there are alternative times and temperatures that give a similar amount of bioburden reduction.

**Gamma** Gamma radiation is a high-energy form of ionizing radiation that is most often sourced by <sup>60</sup>Co. It is believed that gamma radiation inactivates spores by crosslinking proteins and by generation of free radicals when in contact with water. This process can occur at room temperature, though it does require infrastructure to handle and operate a radiation source. Radiation levels that are known to kill most spores is on the order of 2.5 Mrad [28]. Beyond standard spores, *Deinococcus radiodurans* is a hardy organism, whose internal genetic structure is malleable to radiation, making it the one likely survivor on hardware after a solid gamma dose [29]. Dead bodies are unmoved by the gamma process, so an additional approach would be required to remove dead organisms if organic contamination requirements are also a part of the planetary protection considerations for the mission. Scalability is already a reality—several other agencies, including the Department of Defense, use gamma for large-scale sterilization. In fact, during the anthrax mail scare in DC in 2001, the Department of Homeland Security used large-scale gamma radiation

sources to sterilize all incoming mail to the Congress and Executive Office [30].

**γ + Heat (Thermoradiation)** There is a synergistic effect when radiation and heat are combined. Thermoradiation is conducted at lower temperatures, lower radiation doses and an overall shorter process time compared to DHMR or gamma radiation alone to achieve 4-7 logs of reduction for *B. Subtilis* and other spores common to the spaceflight world. Temperatures range from 95-110°C, radiation doses are less than 150 krad and process times are at most 15 hours [31]. This approach may be promising for parts, subsystems or integrated systems that may be able to tolerate common environmental qualification test parameters for temperature and radiation. There are no known studies on spore resistance for this process technique.

While there are other penetrating reduction techniques, such as chlorine dioxide gas and wet heat, those approaches are known to have corrosive interactions with spaceflight hardware and will not be considered here.

#### Summary

Penetrating bioburden techniques are conducted at a range of temperatures from room temperature up to 150°C. Unlike the surface approaches for bioburden reduction, which have a range of bioburden reduction capabilities, the penetrating bioburden techniques as summarized in Table 2, all have the capacity to meet or exceed NASA bioburden requirements with 4-8 log reductions. All of the penetrating bioburden techniques leave residual dead bodies, which may interfere with signal detection, unless combined with a method that mechanically removes microbes from the surface. Like the surface techniques, penetrating techniques should be combined with appropriate precleaning prior to bioburden reduction and perhaps post-reduction approaches that allow for an inert hot gas purge to mechanically move spore carcasses from the hardware.

**Table 2. Summary of Major Penetrating Bioburden Reduction Techniques**

Technique	Log Reduction Range <sup>2</sup>	Possible Spore Resistance?	Residual Dead Bodies
DHMR	2-8	Some	Yes
Gamma	2-8	Some	Yes
γ + Heat	2-8	Unknown	Yes

<sup>2</sup> Ranges show upper and lower bounds, which are process parameter-dependent. DHMR has time-temperature dependence, Gamma is time/dose

dependent.

#### 4. THE WHOLE MOUTH: SYSTEM/SUBSYSTEM-LEVEL BIOBURDEN REDUCTION APPROACHES

All the techniques discussed can be applied to piece parts. The challenge for larger subsystem and even system-level integration is the ability to scale-up a given technique to accommodate larger surface areas, interiors and more complicated and perhaps more diverse geometries than what was seen on smaller size scales.

At the present time, DHMR has been the only technique that has been tested within NASA under scaled up conditions. VHP has been scaled up for use by NIH, CDC and DoD, though for simple geometries such as rooms requiring inactivation of *B. Anthracis* [33]. Additional development will be needed to verify cleanliness of larger complex geometries that are unique to NASA spacecraft. Gamma radiation has been scaled up for use in the food industry as well as by the Air Force. Minor additional tests may be needed to insure penetration through layered metallic structures and at joints and interfaces, which are common to NASA integrated hardware. Foam, Plasma and NO<sub>2</sub> are in their infancy, so the scalability of these tests will need to be fully explored.

**Table 3 Scalability of Techniques With 4-log Reduction Capabilities or Greater<sup>3</sup>**

Technique		Scalability to Spacecraft System Level
<i>Surface</i>	Foam	Needs development
	Plasma	Needs development
	NO <sub>2</sub>	Needs development
	VHP	Yes, up to specific hardware needs
<i>Penetrating</i>	DHMR	Yes
	Gamma	Yes
	γ + Heat	Needs development

#### 5. FLOSSING IN-BETWEEN: INTERFACES AND JOINTS

Surface bioburden techniques have a limited to no role in bioburden reduction at joints and interfaces. The penetrating bioburden techniques show their prowess again, as many of them have the capacity to add an additional level of bioburden reduction when applied at the system level, in addition to what was applied at the subsystem and parts level.

Of the penetrating approaches, DHMR has had extensive research in to the effects of temperature and time on joints and mated surfaces as well as bioburden that may exist in porous media (for example, heatshield and backshell material or o-rings). Work conducted by NASA in the 1960s and 70s

<sup>3</sup> Even though ETO produces a log reduction that is compliant with NASA requirements, due to the environmental implications, ETO will not be

for Voyager, Ranger, Apollo and other missions showed that for three cycles at 145°C , 36 hours each, “there are NO JOINTS OR JOINING TECHNIQUES THAT NEED BE REJECTED SOLELY ON THE BASIS OF INCOMPATIBILITY WITH TERMINAL STERILIZATION. Every type of joining technique can be made acceptable from a sterility standpoint by careful planning and controls.” [34]. Dear reader, we’re not shouting with all-caps here--this quotation was written with the all-caps portion preserved from the original document.

Additional work is needed for evaluating interface bioburden reduction efficacy for gamma and for NO<sub>2</sub>.

#### 6. FRESH BREATH AND CLEAN TEETH: OVERLAPS BETWEEN PP PARAMETERS AND HARDWARE QUALIFICATION ENVIRONMENTS

The overlap of hardware qualification environments and PP bioburden reduction is a bit like the bonus of washing away bacteria and their food sources after you eat by chewing gum (though your real intent for chewing it is to stave off all those onions that you at lunch). Carefully considered, most standard flight qualification environments (thermal, radiation) have overlaps with penetrating bioburden reduction approaches (DHMR and gamma, respectively).

There is an opportunity here for future missions to see planetary protection implementation in a different light—it’s the same, as many of the qualification environments under consideration for Ocean Worlds missions will overlap or in some cases, exceed conditions in time and temperature/dose for at least a 4-log reduction. In addition, current contamination control outgassing bakeouts have time and temperature parameters that are common to DHMR for hardy organisms such as *B. Pumilis* SAFR-32 [35]. Call it what you want—fresh breath (reduced outgassing) or reduced plaque (reduced bioburden), in the end, the application of the technique can be the same.

#### 7. SUMMARY

In summary, this article has broadly surveyed the range of surface and penetrating methods of bioburden reduction. Those methods have been described from a practical point of view: what range of bioburden reduction is expected from a given approach, what limitations in geometry and broad materials compatibility may exist. In particular, considerations need to be made for which techniques leave “dead bodies” behind, which may influence signal to noise differences as they relate to biohazard protocols.

From a spaceflight hardware point of view, we’ve considered the effects of scalability, looking at the ability for a given technique with 3-log reduction or more, to be scaled to a larger size. The Department of Defense and Homeland

included in the discussions that follow.

Security have provided real-world examples of scalability of many of those techniques for bioburden reduction of Anthrax.

In addition to the hardware, the connections between the hardware – joints and interfaces showed from prior NASA efforts in the 1960s and 70s that joints and interfaces require some thoughtfulness, though there is no immediate showstopper to be found.

We conclude this paper with a short summary of incompatibilities for the 4-log techniques described in the article. NASA and the Planetary Science Division will be publically releasing a larger database of parts and materials compatibilities with specific references to sterilization techniques in 2017.

**Table 4: General Incompatibilities for Techniques With 4-log or Greater Reduction Capabilities**

Technique		Examples of Potential Incompatibilities <sup>4</sup>
Surface	Foam	Studies are needed
	Plasma	Non metals etched
	VHP	Conformal coatings at high H2O2 concentrations Unsealed detectors (CCDs, filters, MMICs, etc. Diodes
Penetrating	DHMR	Thin films (grain boundary migration, chemical diffusion and rxns) Joints or interfaces with disparate CTE
	Gamma	Radiation-sensitive electronics Polymers (delam, cracking, oxidation in PE and PTFE)
	$\gamma$ + Heat	Electronics rated for less than 100-150krad and T ~ 95-100°C
	NO <sub>2</sub>	Limited information available.

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<sup>4</sup> The degree of incompatibility is process variable dependent. Users should evaluate the literature, since the process specifics may reveal that the part or

#### REFERENCES

- [1] ISO 14644-1, Classification of air cleanliness by particle concentration (2015).
- [2] For an introduction to the biological terminology referred to in this paper, please see: D.E. Pugel, J.R. Rummel, C.A. Conley, *Tiny houses: Planetary protection-focused materials selection for spaceflight hardware surfaces*. IEEE Aerospace Conference Proceedings (2016).
- [3] A.D. Russell, *Bacterial Spores and Chemical Sporicidal Agents*, Clin Microbiol Rev, **3**, 99-119 (2002).
- [4] A.H. Love, C.G. Bailey, M.L. Hanna, *et al. Efficacy of liquid and foam decontamination technologies for chemical warfare agents on indoor surfaces*. J. Hazard Mater. 196, 115–122 (2011).
- [5] J. Guan, M. Chan, BW Brooks, L Rohonczy, *Influence of temperature and organic load on chemical disinfection of Geobacillus stearothermophilus spores, a surrogate for Bacillus anthracis*, The Canadian Journal of Veterinary Research **77**,100–104 (2013).
- [6] J Zhang *et al.* Sterilization Using High Pressure Carbon Dioxide – A Review, J. Supercrit. Fluids, **38**, 354 (2006).
- [7] E Shieh *et al.* Sterilization of Bacillus pumilus spores using supercritical fluid carbon dioxide containing various modifier solutions, J Microbiol Methods., **76**, 247-52 (2008).
- [8] J Zhang, TA Davis, MA Matthews, MJ. Drews, *et al.*, Sterilization using high-pressure carbon dioxide An J. of Supercritical Fluids **38**, 354–372 (2006).
- [9] IL Shechmeister, *Sterilization by ultraviolet irradiation*. In: Block SS, ed. Disinfection, sterilization, and preservation. Philadelphia: Lea & Febiger, 1991: 553-65.
- [10] S. Osman *et al.* Effect of Shadowing on Survival of Bacteria under Conditions Simulating the Martian Atmosphere and UV Radiation, Appl. Environ. Microbio. **74**, 959-970 (2008).
- [11] M.R. Tirumalai, *et al.*, Candidate Genes That May Be Responsible for the Unusual Resistances Exhibited by Bacillus pumilis SAFR-032 Spores, PLOS One, **8** e66012 (2013).
- [12] P.A. Vaishampayan, E. Rabbow, Gerda Horneck, and Kasthuri J. Venkateswaran. *Survival of Bacillus pumilus Spores for a Prolonged Period of Time in Real Space Conditions*, Astrobiology, **12**, 487-497 (2012).

material is actually compatible for that set of process variables.

- [13] P Setlow, *Spores of Bacillus subtilis: their resistance to and killing by radiation, heat and chemicals.*, J Appl Microbiol., **101**, 514–525 (2006).
- [14] P. Setlow, *Spore Resistance Properties*, Microbiol. Spectr. **2**, (2014).
- [15] S. Farquharson, AD Gift, P Maksymiuk, and FE Inscore, *Rapid dipicolinic acid extraction from Bacillus spores detected by surface-enhanced Raman spectroscopy*. Appl. Spectrosc. **58**, 351–354 (2004).
- [16] J Sawai *et al.* *Heat activation and germination-promotion of Bacillus subtilis spores by infrared radiation*, Int. Biodeter. & Biodeg. **63**, 196-200 (2009).
- [17] J Ehlbeck, *et al.* *Low temperature atmospheric pressure plasma sources for microbial decontamination* J. Phys. D: Appl. Phys. **44**, 013002 (2011).
- [18] M Cooper *et al.* *Decontamination of Surfaces From Extremophile Organisms Using Nonthermal Atmospheric-Pressure Plasmas*, IEEE Transactions on Plasma Science, **37**, 866-871 (2009).
- [19] TG Klampf, *Cold Atmospheric Air Plasma Sterilization against Spores and other Microorganisms of Clinical Significance* Appl Environ Microbiol, **78**, 5077-5082 (2012).
- [20] S Moreau, M Moisan, *et al.* *Using the flowing afterglow of a plasma to inactivate Bacillus subtilis spores: Influence of the operating conditions*. J Appl Phys., **88**, 1166-1174 (2000).
- [21] HW Herrmann, I Henins, J Park, GS Selwyn, *Decontamination of chemical and biological warfare (CBW) agents using an atmospheric pressure plasma jet (APPJ)*, Phys. Plasmas, **6**, 2284 (1999).
- [21] K Ishizaki, N Shinriki, & H Matsuyama, *Inactivation of Bacillus spores by gaseous ozone*. Journal of Applied Bacteriology, **60**, 67-72 (1986).
- [22] Centers for Disease Control, *Guideline for Disinfection and Sterilization in Healthcare Facilities*, (2008).
- [23] RG Rice, *Ozone and anthrax—Knowns and unknowns*. Ozone: Science and Engineering, **24**, 151-158 (2002).
- [24] SF Chou, *et al.*, *Evaluation of the Effects of Hydrogen Peroxide on Common Aviation Structural Materials*, FAA Report DOT/FAA/AM-09/23 (2009).
- [25] European Cooperation for Space Standardization, *Vapour phase bioburden reduction for flight hardware ECSS-Q-ST-70-56C*. Note: NASA has accepted this standard for its own use.
- [26] A Mehta, PS Sequeria, G Bhat, *Bacterial contamination and decontamination of toothbrushes after use*, NY State Dental J, **73**, 20-2 (2007).
- [27] European Cooperation for Space Standardization, *Dry heat bioburden reduction for flight hardware*, ECSS-Q-ST-70-57C (2013). Note: NASA has accepted this standard for its own use.
- [28] ANSI/AAMI ST32, *Guideline for Gamma Radiation Sterilization*. Arlington, VA, 1991.
- [29] MM Cox and JR Battista *Deinococcus radiodurans – the consummate survivor*, Nature Reviews Microbiology **3**, 882-892 (2005).
- [30] MF Derosiers, *Irradiation applications for homeland security*, Radiation Physics and Chemistry, **71**, 479-482 (2004).
- [31] *Advances in Sterilization and Decontamination: A Survey*, NASA Report SP-5105 (1978).
- [32] AA Poliakov *et al.* *Bactericidal action of nitrogen dioxide on the vegetative and sporous forms of Bacillus anthracis*. Mikrobiolohichnyy Zhurnal, **24** 43-45 (1962).
- [33] For example, VHP scaled up for use for a C-1141 aircraft. MD Brickhouse, *et al.* *Vaporous Hydrogen Peroxide Decontamination of a C-141B Starlifter Aircraft: Validation of VHP and Modified VHP Fumigation Decontamination Process via VHP Sensor, Biological Indicator and HD Simulant in a Large-Scale Environment*, Edgewood Chemical and Biological Technical Center Technical Report ECBC-TR-510, (2007).
- [34] *Advances in Sterilization and Decontamination: A Survey*, NASA Report SP-5105 (1978).
- [35] . For example, the REMS instrument on the Curiosity rover: J. Gomez-Elvira *et al.*, *REMS: The Environmental Sensor Suite for the Mars Science Laboratory Rover*, Space Science Reviews, **170** 583-640 (2012).



## BIOGRAPHY



**D.E. (Betsy) Pugel** received her B.S. in physics from the University of Michigan and Ph.D. in experimental condensed matter physics with a focus on the interaction between metal oxide surfaces and ultraviolet spectroscopy from the University of Maryland-College Park. Since

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**John D. Rummel** received a B.A. in Environmental Biology from the University of Colorado, Boulder, in 1974. After serving as a Naval Flight Officer he began his dissertation research at Stanford University, and earned his doctorate in evolutionary ecology in 1985, the same year he joined

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**Catharine Conley** is NASA's Planetary Protection Officer. Conley's research at NASA Ames Research Center focused on the biochemistry and evolution of muscle tissue, the basis for motility in all multicellular organisms. A recent offshoot of this work explores the adaptation of multicellular organisms to extreme environments,

including the Atacama Desert in South America and the Arctic tundra. Dr. Conley has been involved in several spaceflight experiments using the nematode worm *Caenorhabditis elegans*, the first of which was flown on the last mission of the Space Shuttle Columbia. Flight hardware was recovered after the tragic accident, and when opened it was seen that the spaceflown experimental animals were still alive, a finding of considerable relevance to Planetary Protection. In 1999, Dr. Conley joined NASA after completing postdoctoral studies at the Scripps Research Institute in La Jolla, CA, where she characterized a family of proteins involved in regulating the actin cytoskeleton that are required for proper muscle contraction. Dr. Conley received a Ph. D. in Plant Biology in 1994 from Cornell University in Ithaca, NY, where her graduate research focused on characterizing functional defects in petunias that are mutant for male sexual reproduction. She earned two B.S. degrees from the Massachusetts Institute of Technology, one in Life Sciences and one in Humanities, involving a major in Russian and French languages and a minor in music performance.

