TRACK Number

Brushing Your Spacecraft's Teeth:

A Review of Bioburden Reduction Processes for Planetary Protection Missions

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Outline

- Introduction: Why Brush Your Spacecraft's Teeth?
- Brush and Swish
- Deep Cleaning
- The Whole Mouth
- Flossing In-Between
- Fresh Breath and Clean Teeth

Outline

What this is:

- An orientation guide to the world of biology for planetary protection missions
- A different way to think about the materials selection process for planetary science missions.

What this isn't:

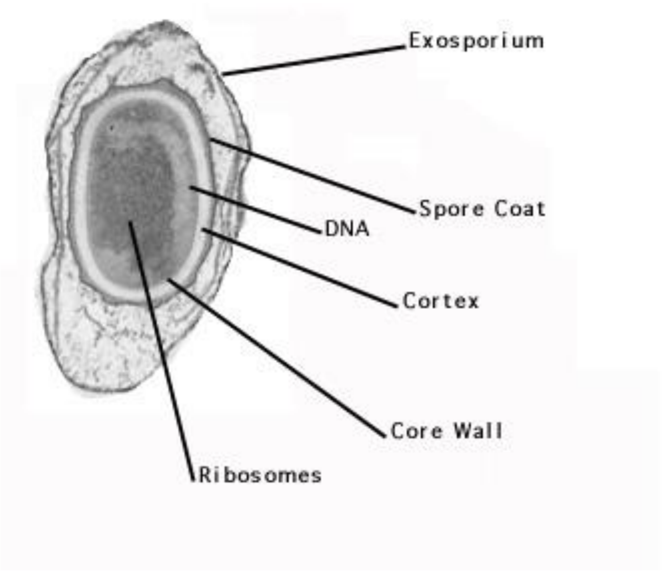
- A review of planetary protection policy
- A Headquarters-directed list of what can or cannot/should or should not be used.

Introduction

Planetary Protection: NASA planetary science missions are required to comply with requirements that protect the science and protect the planet, depending on the mission's objectives.

Why Brush Your Spacecraft's Teeth?

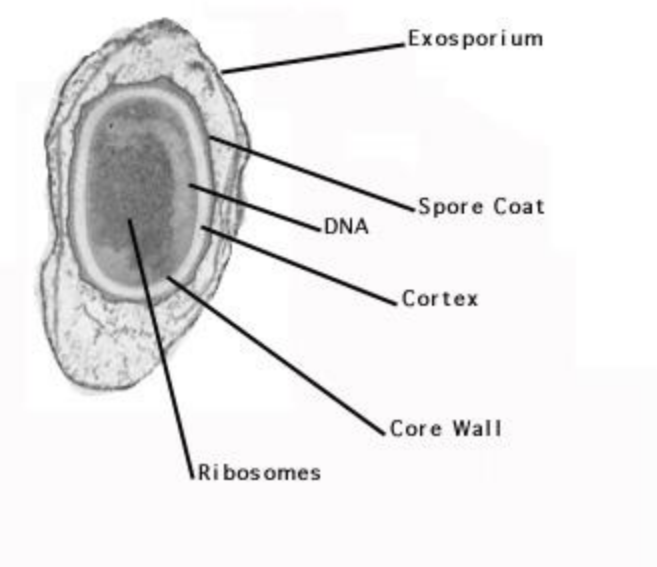
Most cleanrooms exist at 70F/20C and 50% humidity.



Why Brush Your Spacecraft's Teeth?

Take a census by counting.

Counting What?
Counting Spores.



Bacteria can form a metabolically inactive state when environmental conditions are harsh.

When we take a census, we heat shock the collected bacteria to drive spore formation.-- looking for the hardiest critters, those that could potentially survive the trip to another place in our solar system.

Log Reduction

“Log Reduction” is not about chopping firewood…

START With a known level of spores
You know this level because you’ve sampled and assayed.

SELECT A bioburden process that has been well characterized (e.g. NASA Standard Process)

APPLY that process to your hardware with appropriate level of controls.

END RESULT: a number of logs of reduction in spores

Methods of Bioburden Reduction

Methods of bioburden reduction fall into two main camps:

- **Surface**
- **Penetrating**

Surface

- Addresses exterior portions of hardware only
- Can include: piece parts, components prior to integration

Common Methods:

- Physical Removal
- Irradiation (UV, IR)
- Reactive Chemical Species (VHP)

NASA Standard Approaches:

- Physical Removal
- Vapor Hydrogen Peroxide

Penetrating

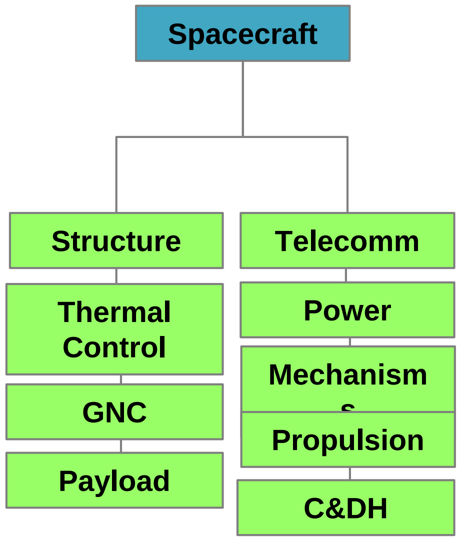
- Addresses interior portions of hardware
- Can include: nested, porous or integrated structures

Common Methods:

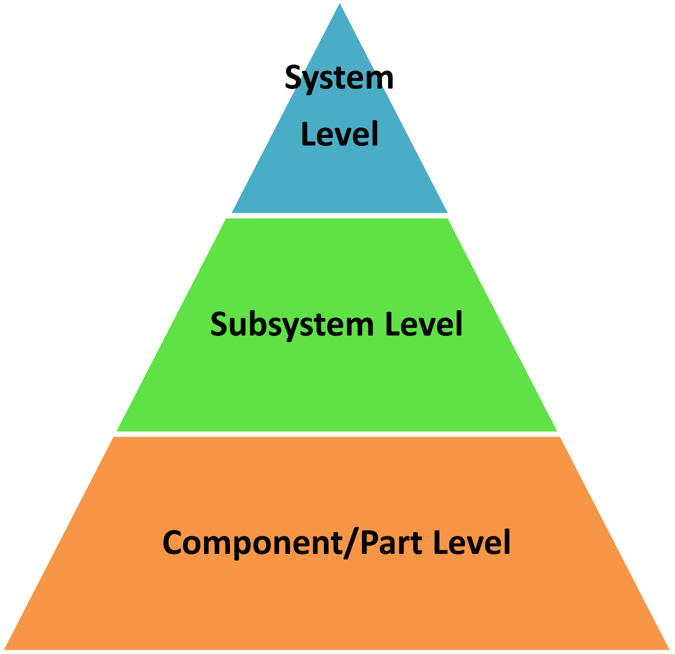
- Dry Heat Microbial Reduction (DHMR)
- Gamma Irradiation
- Gamma + Heat
- Autoclaving

NASA Standard Approaches:

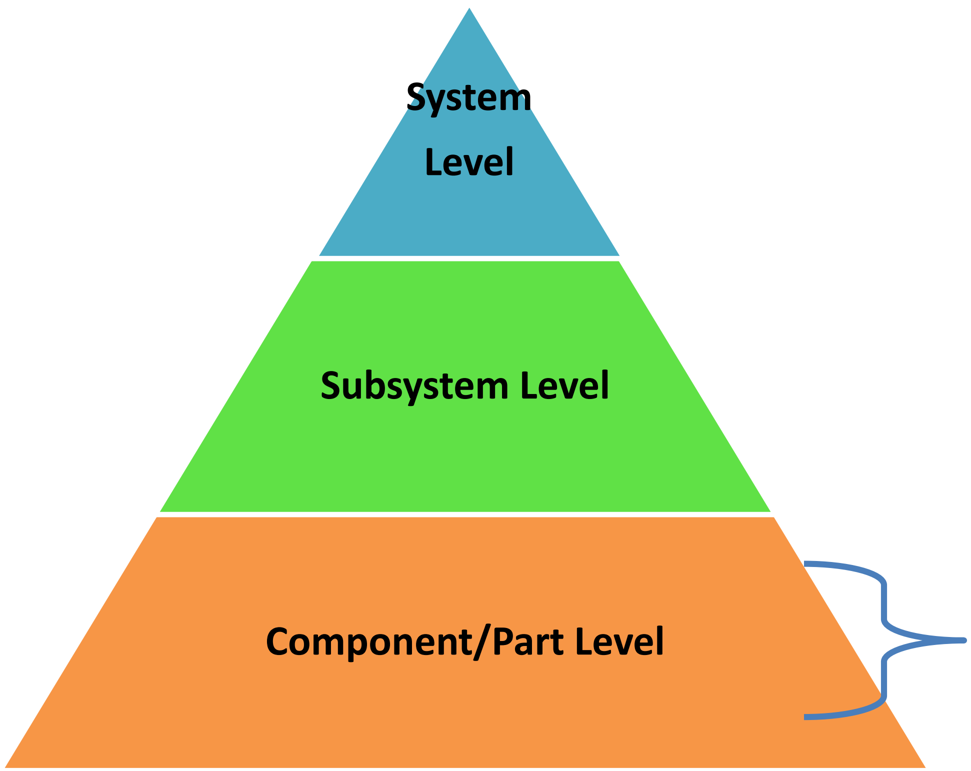
- Dry Heat Microbial Reduction



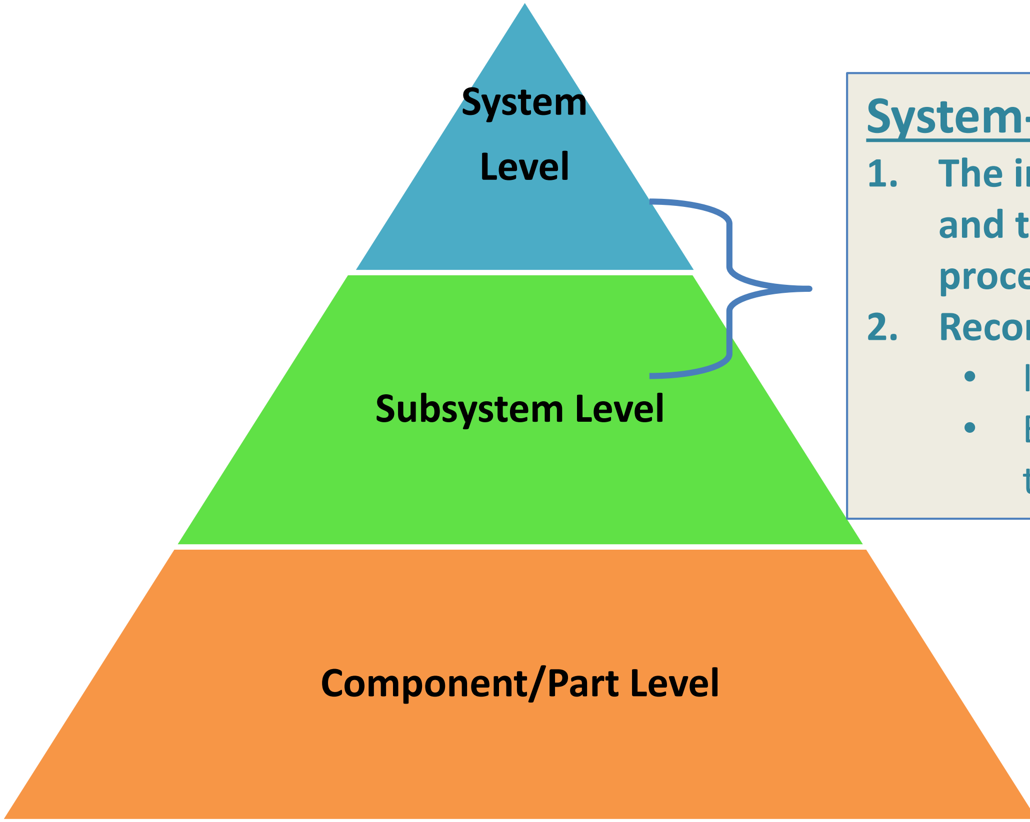
Spacecraft
Structure
Thermal Control
GNC
Payload
Telecomm
Power
Mechanisms
Propulsion
C&DH



System Level
Subsystem Level
Component/Part Level



- **The Stuff:** Materials and Piece Parts compatible with protocols/processes
- **Cleaning the Stuff:** Protocols/Processes to reduce bioburden



<u>System-Subsystem Level</u>:

1. **The interfaces between parts and subsystems and their tolerances to bioburden reduction processes**
2. **Recontamination prevention:**
   - Internal to the system
   - Between the internal and external worlds of the system

How do we pick a process?

- Materials Compatibility
- Scalability? Piece Parts bioburden reduction? Full-spacecraft?
- Recipe? Is there a NASA standard process? Has NASA accepted someone else's process (ESA)?

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Brush and Swish: Surface Bioburden Reduction Techniques

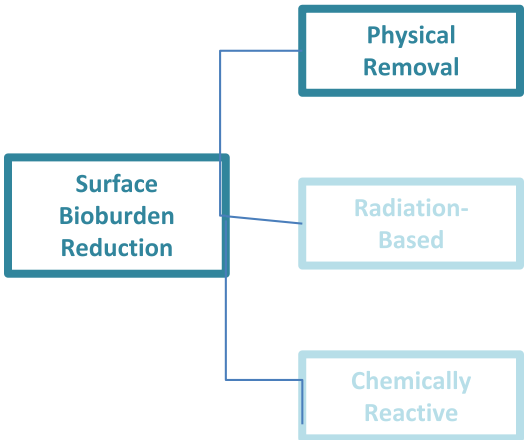
- Surface Bioburden Reduction
  - Physical Removal
  - Radiation-Based
  - Chemically Reactive

Brush and Swish: Surface Bioburden Reduction Techniques

Surface Bioburden Reduction

- Physical Removal
- Radiation-Based
- Chemically Reactive

Brush and Swish:
Surface Bioburden Reduction Techniques



Physical Removal

- Solvents
- Foams
- Carbon Dioxide

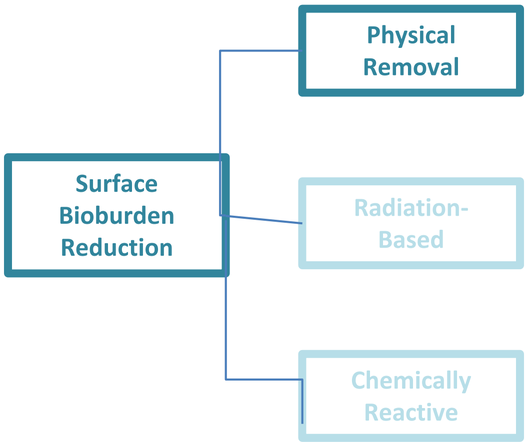
Solvents

- Often the first line of defense
- Easily accessible
- Work by dissolving or moving surface inhabitants
- Often paired with additional physical removal via wiping and or ultrasound
- Influenced by: hardware surface geometry, surface energy of the solvent, microbial adhesion energy
- A single solvent doesn't always lead to the same log reduction

Sporicidal Solvents:

- Majority of solvents are not sporicidal. (e.g. IPA , acetone, methanol don't kill spores)
- Sporicidal solvents: glutaraldehyde, H_2O_2, ethylene oxide, chlorine & iodine solvents

Brush and Swish: Surface Bioburden Reduction Techniques



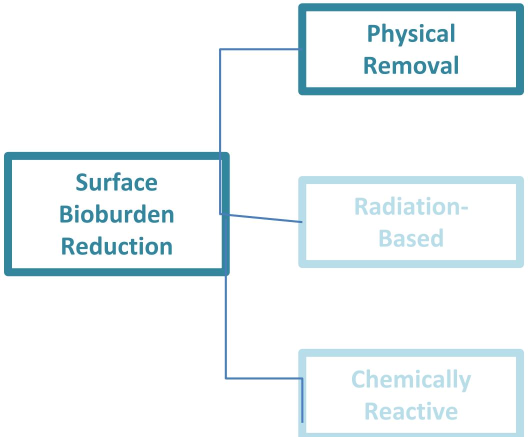
Physical Removal

- Solvents
- Foams
- Carbon Dioxide

Foams

- Commonly used for decontamination of large areas
- Physical nature of foam allows for penetration into various geometries on the size scale of a foam bubble
- Influenced by: the starting biological load on a piece of spaceflight hardware
- Limited studies are available at present, so long-term effects and spore resistance is unknown..
- Scalability? Possibly, due to the physical nature of foams.
- Recipe:? No NASA/ESA standard process.

Brush and Swish:
Surface Bioburden Reduction Techniques



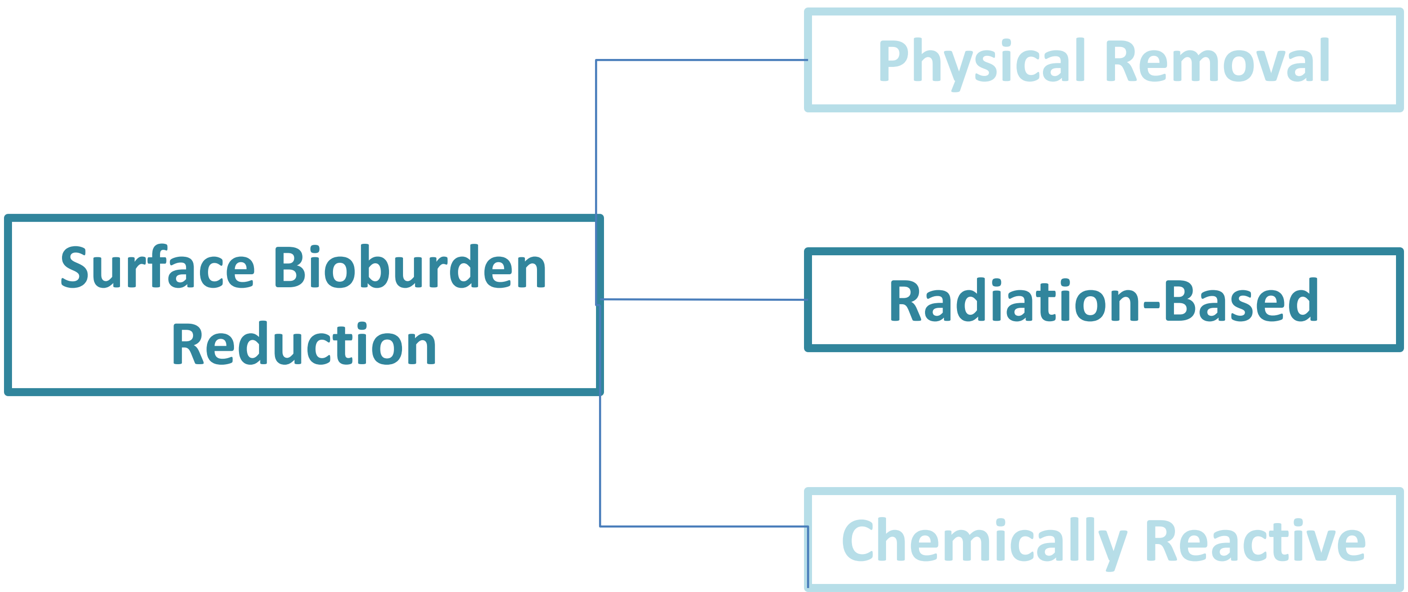
Physical Removal

- Solvents
- Foams
- Carbon Dioxide

Carbon Dioxide

- Often employed n the medical and food industry when there are heat- or chemical sensitivity concerns
- Solid CO_2 can be delivered as a particulate so as to bead blast the surface of hardware (CO_2 snow)
- Not effective for spore inactivation, though it is effective for particle removal.
- Majority of work in the literature points to coupling CO_2 with a sporecide or sterile filter
- No NASA/ESA approved processes for use.

Brush and Swish:
Surface Bioburden Reduction Techniques



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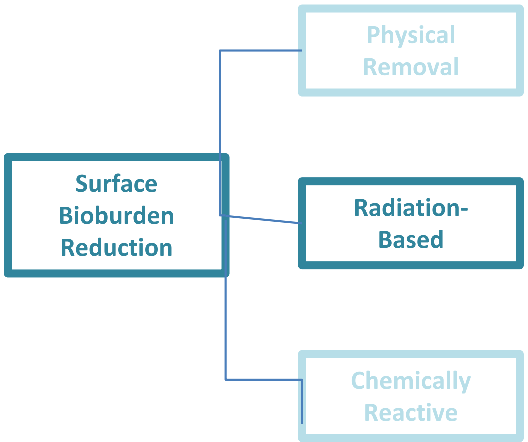


Radiation-Based

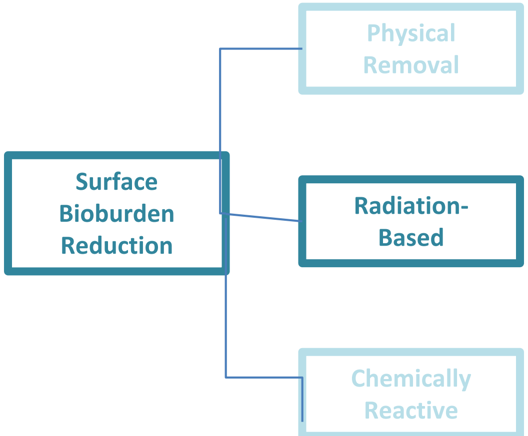
- Ultraviolet
- Infrared

Ultraviolet (UV)

- Relies on breakdown of microbial DNA at 254 nm. Breakdown results in microbes being unable to reproduce/grow
- Penetration depth is so short that even a layer of spores is sufficient to protect a layer of spores beneath it from harm
- Influenced by: Shadowing, geometry, source-hardware distance, initial level of surface contamination. Cannot be used for interiors or holes
- Can only reach by direct exposure. Scattering length is short
- Spores are able to develop resistance to UV over time.
- Scalability? Possibly
- Recipe:? No NASA/ESA standard process, as results are a mixed bag

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).

Brush and Swish: Surface Bioburden Reduction Techniques



Radiation-Based

- Ultraviolet
- Infrared

Infrared (IR)

- Relies on local thermal degradation of the spore coat and internal spore contents
- Influenced by: Shadowing, geometry, source-hardware distance, initial level of surface contamination. Cannot be used for interiors or holes
- Can only reach by direct exposure.
- Mixed results reported in the literature: some reports show increased germination when exposed to IR [J Sawai *et al. Heat activation and germination-promotion of Bacillus subtilis spores by infrared radiation*, Int. Biodeter. & Biodg. **63**, 196-200 (2009)]
- Scalability? Possibly
- Recipe:? No NASA/ESA standard process, as results are a mixed bag

Brush and Swish:
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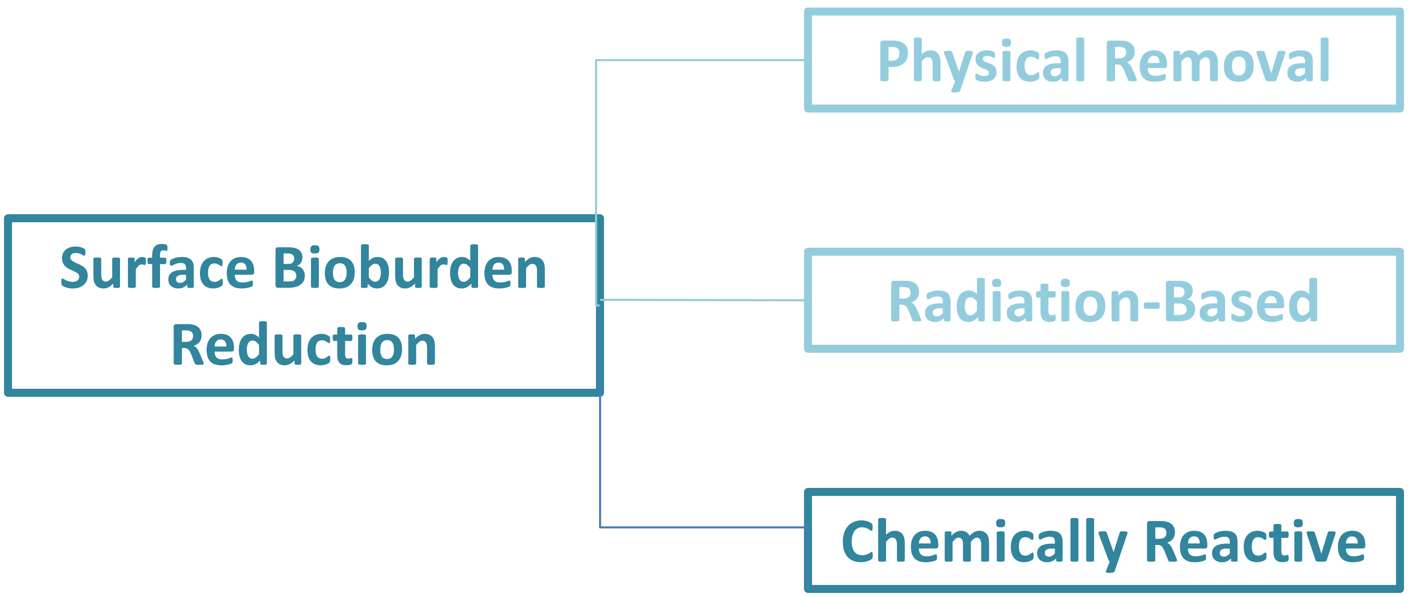

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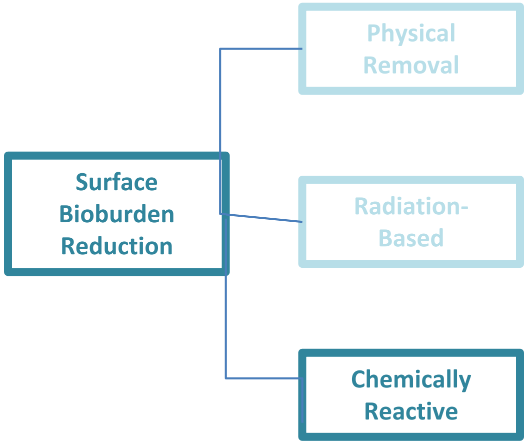


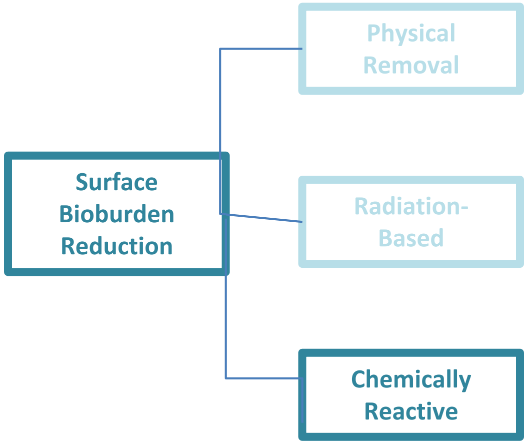
Chemically Reactive

- Plasma
- ~~Ethylene oxide~~
- Nitrogen Dioxide
- Ozone
- Hydrogen Peroxide

Chemically Reactive Methods

- Relies upon reactive species that are able to disrupt the spore coat and enter into its core to destroy it.
- Often employed in situations where materials would be intolerant of bioburden reduction via techniques that employ high temperatures (DHMR) or humidity (autoclaving)
- Requires some awareness of corrosion susceptibility and etch rates
- Ethylene oxide was popular for NASA use in the 1960s/1970s, but fell out of use due to the Clean Air Act in 1995 (ETO is paired with a Chlorofluorocarbon), so we won't discuss this here.

Brush and Swish:
Surface Bioburden Reduction Techniques



Chemically Reactive

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Plasma

- Partially ionized gas composed charged (ons/radicals/electrons) + uncharged species
- Spores are killed via charged species which disrupt the spore coat
- Currently no studies on resistance
- Scalability to large sizes: Potentially, limited by beam size/ rastering capability
- Recipe? No NASA/ESA standard process

Brush and Swish:
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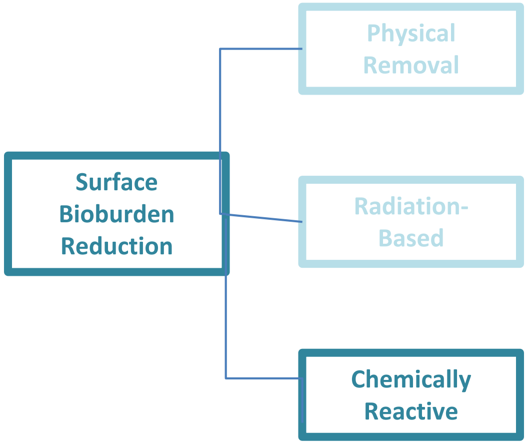

Chemically Reactive

- Plasma
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Nitrogen Dioxide

- Reactive species of NO_2 gas degrades spore coat
- Can be generated at room temperature
- Cycle times ~ minutes for 6-8 log reduction (*G. stearothermophilus)*
- Resistance: Unknown, limited amount of work done
- Scalability to large sizes: Potentially, limited space in which gas may diffuse
- Recipe? No NASA/ESA standard process

AA Poliakov et al.. Mikrobiolohichny Zhurnal, ***24*** *43-45 (1962).*

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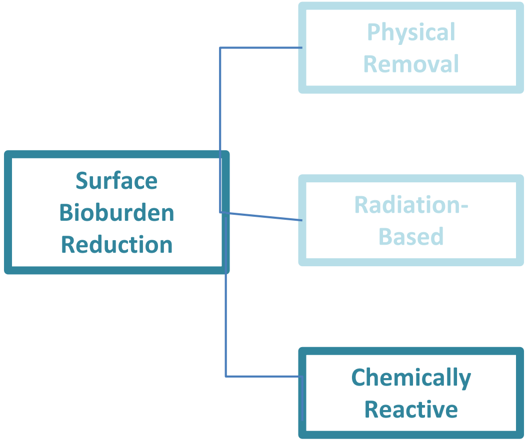

Chemically Reactive

- Plasma
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- Ozone
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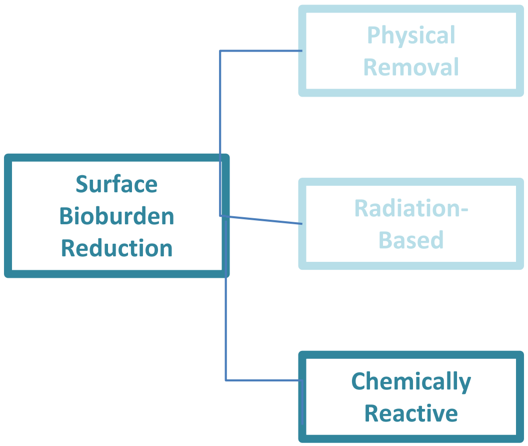
Ozone

- Reactive species of oxygen degrades outer coat of spore and inner core.
- Maybe a suitable approach for tool sterilization, as commercial systems are available
- No NASA/ESA standard, but could apply use for tools

Brush and Swish:
Surface Bioburden Reduction Techniques



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- Ozone
- Hydrogen Peroxide

Hydrogen Peroxide

- Vapor Hydrogen Peroxide (VHP) generated by thermal vaporization or pulled by pressure using a carrier gas into a vacuum chamber
- Good materials compatibility for metals and nonmetals
- Scalability? Limited by chamber size
- Recipe? NASA has accepted ESA standard process for use.

Summary:
Surface Bioburden Reduction Techniques

Pro Tips

- Clean before you reduce! Without appropriate pre-cleaning, surface bioburden techniques are not as effective as they may seem. (shadowing by organic/particulate matter).

- Reusing mouthwash or a dirty tooth brush is disgusting at many levels…Don't recycle solvents or gases reuse. Take care to use ovens/vacuum chambers that have been cleaned or handled with the utmost care for recontamination!

- Bioburden reduction techniques are not additive: 1-log reduction by one process+ 1-log reduction by another process does not equal a 2-log reduction!

Brush and Swish: Surface Bioburden Reduction Techniques

Technique	Log Reduction Range	Possible Spore Resistance?	Residual Dead Bug Bodies
Solvent	NA	Possible	Partially
Foam	4	Unknown	Partially
Ultraviolet	< 2	@low water activity	Yes
Infrared	2-6	Unknown	Yes
Super CO_2	< 1/None	NA	Partially
NO_2	4-8	Unknown	Yes
Plasma	2-4	Unknown	Yes
ETO	4	Yes	Partial-none
VHP	4-6	Yes	Partial-none

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NASA Standard Approaches:

- Dry Heat Microbial Reduction

Deep Cleaning: Penetrating Bioburden Reduction

Dry Heat Microbial Reduction

- NASA standard process
- T=110-200C for extended durations (e.g 110°C for 50 hours)
- Some organisms are known to be hardy to lower temp/time, leading to spec revisition.
- Overlaps exist between DHMR specs and specs for MIL-SPEC 810f-rated parts

Gamma Radiation

- Inactivation of spores is thought to occur via crosslinking of proteins and generation of radicals when in contact with water
- Room temperature process, though 2.5MRad is what is needed for most spores.

Gamma + Heat

- Takes advantage of synergy between gamma and heat
- Alternative to DHMR and Gamma, when some parts may be able to withstand some heat and some radiation but not at the levels of standard DHMR and known gamma
- Temperatures = 95-110C and radiation < 150 krads
- 4-7 log reduction occurs in < 24 hours
- May be promising for integrated systems that can withstand standard qualification environments
- Spore resistance is unknown

Advances in Sterilization and Decontamination: A Survey, NASA Report SP-5105 (1978).

Deep Cleaning: Penetrating Bioburden Reduction

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

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The Whole Mouth: System-Level Bioburden Reduction

Say Ah!

- What we have seen is applicable to piece-parts
- Scaling up to a system-level approach requires work!
- DHMR is the only approach currently approved for system-level bioburden reduction
- The others have promise (e.g. gamma)

Technique		Scalability to Spacecraft System Level
Surface	Foam	Needs development
	Plasma	Needs development
	NO_2	Needs development
	VHP	Yes, up to specific hardware needs
Penetrating	DHMR	Yes
	Gamma	Yes
	γ + Heat	Needs development

http://solarsystem.nasa.gov/galleries/viking-in-the-oven

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Joints and Interfaces

- Much like how mouthwash has a limited role in getting to the gunk between your teeth, surface bioburden reduction techniques have a limited to zero role in bioburden reduction at joints and interfaces.
- Penetrating approaches such as DHMR have been shown to be effective
- Gamma requires additional work

Fresh Breath and Clean Teeth

It's possible to take advantage of hardware environmental qualification tests for bioburden reduction purposes: the bonus of fresh breath that you get after brushing your teeth!

Parts-Level: Qualification Testing for Europa Environments (Radiation and T):

Couple radiation and temperature qualification tests with sterilization qualification testing

- EEE Parts Qualification
- Structures/Materials Testing

Subsystem/Instrument/System-Level:

Thermal- I&T Campaign Testing:

Couple radiation and temperature qualification tests with sterilization qualification testing

- **TVAC**
- **Contam Bakeouts**

Keep Our Solar System Weird

www.planetaryprotection.nasa.gov

Backup Slides

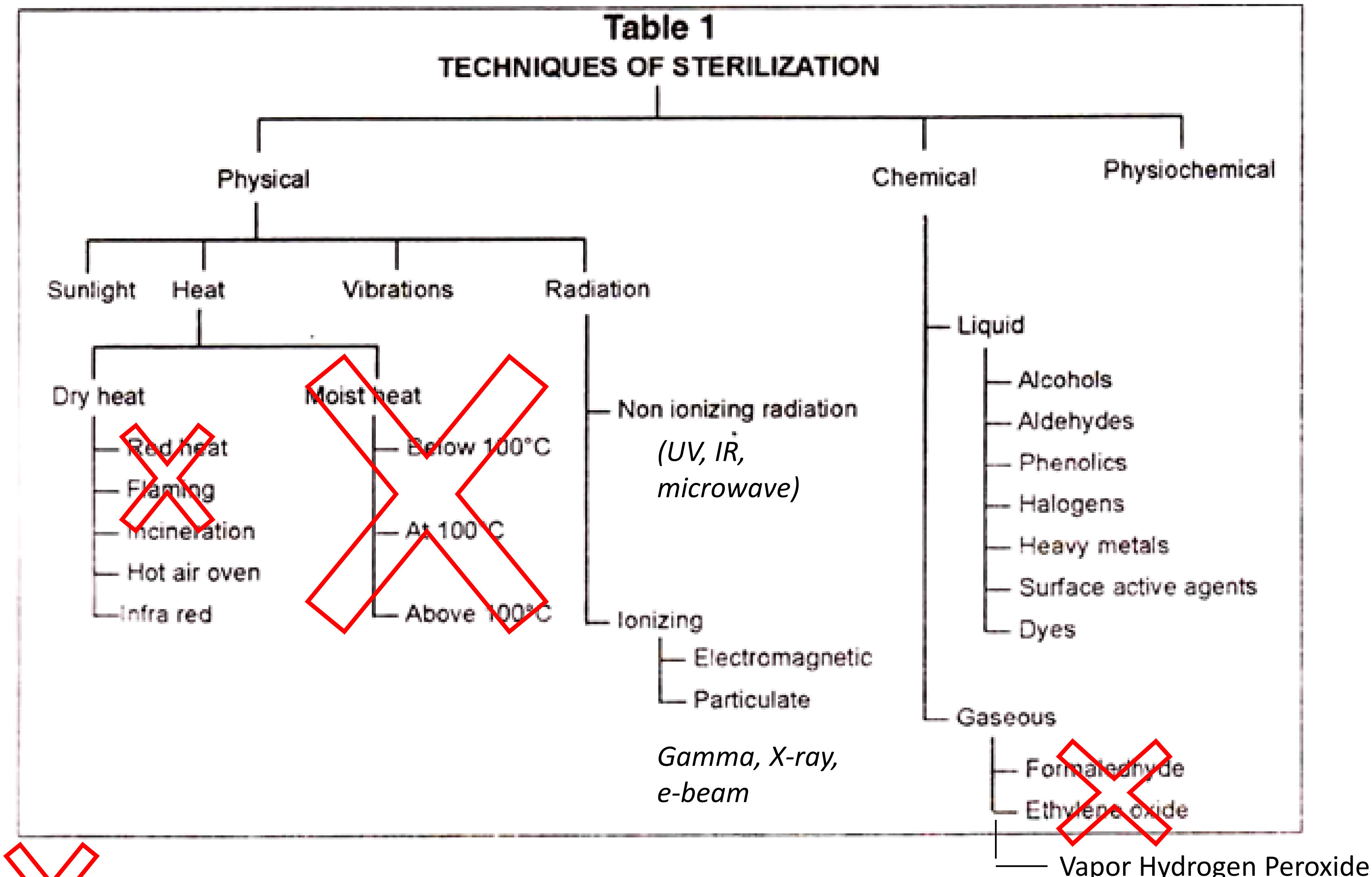
An unconscious tribute to Kanye West…*PP Processes*

Tested	**Heat**: NASA has specifications for time and temperature *Agency-level testing in the 60s and 70s; project-specific data from the 70s onwards (not public)* **Vapor Hydrogen Peroxide:** NASA accepts ESA's specs for time-temp-pressure *ESA has compatibility data* **CO_2 Snow Cleaning:** ESA has time-pressure specs formally accepted by NASA *ESA has compatibility data* **Gamma:** No specifications from NASA or ESA *Limited compatibility data from NASA from the 60s and 70s*
Invested	**Plasma Jet Sterilization:** Phase I SBIR, Eagle Harbor Technologies **CO2 Snow and Plasma:** Geometric Effects, some materials compat, PPO-funded **Laser-Induced Plasma Shockwave:** Efficacy and Geometric Effects, PPO-funded **Vapor Hydrogen Peroxide**: Electronic Packaging Effects, PPO-funded
Unexplored, but interested…	**Electron beam** **Ozone** **Nitrogen Dioxide** **X-ray**: Very limited testing
Retro, chango and maybe a go? Or maybe a no?	**Terminal Sterilization by use of thermite**: explosive sterilization at EoM: Goddard **Iodine Marking of spacecraft:** Messy! SETI

OK, Kanye, but we're not alone…

USDA FDA CDC NIH HHS DHS	**Heat**: Majority are steam heat implementations (vs. dry heat) **CO_2 Snow Cleaning:** Not used by others **Gamma:** A lot of research by CDC, but no specs: Cost-prohibitive for them (but maybe not for us) ***References for the curious:*** *CFR Chapter 21 – Part 110 (USDA, Food)* *CDC Guidelines for Disinfection/Sterilization* *https://www.cdc.gov/hicpac/pdf/guidelines/Disinfection_Nov_2008.pdf*
Military	**MIL SPEC 810 Rev G, Method 501.5-** Procedure I nonoperational, high temperature testing and Procedure III – operational at room temperature, after high temperature testing Temperature Range: -40° to +125°C, nonoperational Duration Equivalent to DHMR **MIL 5090 : High Temperature Adhesives** **MIL : High Temperature Structures for Airframes** **Vapor Hydrogen Peroxide :** Use for anthrax, sans organic contam.
Automotive Industry	**Automotive Electronics Qualified Parts AEC-Q100, -Q101, -Q200:** Temperature Range: -40°C to +125°C, operational (!) Microcircuits, Discrete Semiconductors and Passive Components AEC-Q100 is a set of reliability stress tests to qualify integrated circuits for automotive applications

What others do that NASA won't…



X = Tested and not used by NASA due to compatibility/lifetime issues