BIOREDUCTION OF SOLID ROCKET MOTORS FOR PLANETARY PROTECTION

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ABSTRACT

Solid rocket propulsion systems have been used for in-space applications including planetary exploration missions for many years. Current NASA science lander projects require solid rocket propulsion systems to touchdown on the surface of potentially life-supporting planets and moons. A critical requirement of these missions is the prevention of accidental transportation of Earth's microbes to these environments. This mission requirement places an increased importance on the ability to reduce the biological burden that may be on board the solid propulsion systems and potentially deposited in a habitable environment. Some traditional interplanetary spacecraft decontamination operations could reduce the reliability of the solid propulsion system, indicating a need for new decontamination procedures. New techniques for biological burden reduction are being studied and may become the method of choice to ensure adequate reduction has been achieved. These techniques include biocidal elimination through chemical agents already present within the motor and cellular disruption due to assembly and operational environments induced in the motor. Recent investigations into the effectiveness of these techniques have generated promising experimental results. These techniques and current experimental results will be presented.

NOMENCLATURE

CFU	Colony Forming Units
DHMR	Dry Heat Microbial Reduction
DOS	De-Orbit Stage
I&T	Integration and Test
MLI	Multi-Layer Insulation
NASA	National Aeronautics and Space Administration
NCBI	National Center for Biotechnology Information
RNA	Ribonucleic Acid
Sa	Arithmetical Mean Height
SAE	Society of Automotive Engineers
SEP	Separation
SRM	Solid Rocket Motor
Str	Texture Aspect Ratio
TBD	To Be Determined
TSA	Trypticase Soy Agar
TSB	Trypticase Soy Broth
VHP	Vaporized Hydrogen Peroxide Purges

INTRODUCTION

The National Aeronautics and Space Administration (NASA) mission is to explore new frontiers [1]. Space is full of the unknown and NASA seeks to further understand the universe and search for life on other planets. In the next few decades missions are planned to explore Europa, one of Jupiter's many moons. The surface of Europa is covered in an ice crusted water ocean making it a possible habitat for life. The Europa Clipper mission will place a spacecraft in orbit around Europa. The goal of this mission is to analyze the surface of Jupiter's moon and aid in determining whether life could reside there [2]. The Europa Lander mission will send scientific instruments to the Europan surface to take measurements and search for life or bio-signatures of life. All exploration missions pose many challenges, one of the largest being planetary protection.

Planetary protection requirements stem from 1958 international treaty in which all countries on the treaty agree to conduct exploration in a manner that does not contaminate the visited extraterrestrial bodies [3]. NASA has set requirements for each mission category from I to V. A lander mission on a planetary body with the possibility of life is classified as category IV [4]. This means that the Europa Lander mission must limit the probability of contamination to no more than $1x10^{-3}$ during the entire period of exploration (50 years after landing on the surface) [5].

Planetary protection poses a particularly challenging problem due to the complexity of exploration missions. Most bioreduction techniques were developed for the NASA's Viking 1 and 2 lander missions to Mars and mostly focused on heat sterilization techniques [6]. Newer techniques have been developed, one of which is vaporized hydrogen peroxide purges (VHP). The process of VHP consists of placing the object in a sealed compartment and introducing vaporized hydrogen peroxide to eliminate microbes. Although both of these approaches are effective, they can be harmful to certain components of a spacecraft, in particular the solid rocket motor (SRM) on the de-orbit stage (DOS) of the Europa Lander mission. The following sections will examine the challenges of historical bioreduction processes with SRMs, the strategy to sufficiently bioreduce the SRM for the DOS of the Europa Lander, and the experiments conducted to support the proposed strategy.

RESULTS AND DISCUSSION

HISTORIC PLANETARY PROTECTION METHODS

All missions must comply with planetary protection requirements leading to different strategies of compliance. One of the first that underwent planetary protection procedures is the NASA Viking Project missions to Mars. Viking 1 was the first lander sent to another planet and was launched in 1975 to look for life on Mars. Because of its mission, strict planetary protection requirements were put into place to not contaminate the Martian biosphere with earth microorganisms [7]. The Viking lander components are shown in Figure 1.

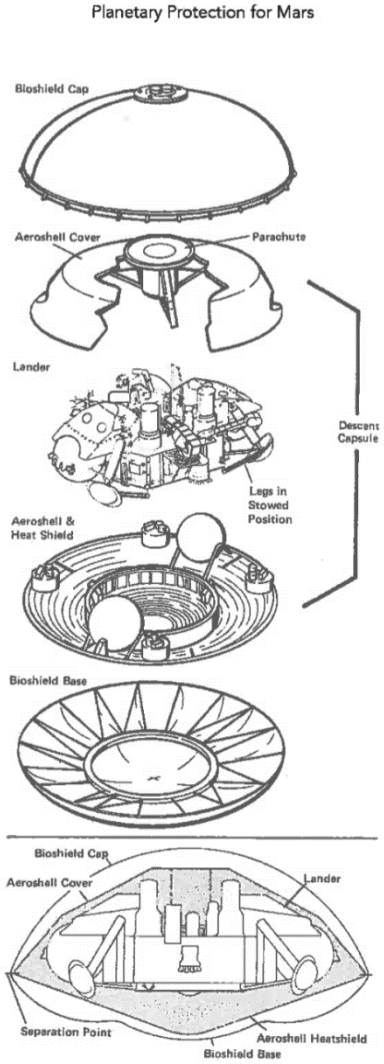

*Figure 1: Components of Viking Lander in hermetically sealed capsule [**7**].*

The Viking missions took four steps in their approach to meet the planetary protection requirements [7].

1. Use materials that can withstand thermal sterilization techniques
2. Use manufacturing processes to minimize biological build up

3. Use heat sterilization techniques on to components and assemblies

4. Conduct final sterilization and hermetically seal the lander in a capsule to maintain sterilization

Viking relied heavily on thermal sterilization also known as dry heat microbial reduction (DHMR). DHMR involves heating the motor to a specified temperature, above 105 °C, for a specified amount of time to achieve bioreduction.

PLANETARY PROTECTION FOR SOLID ROCKET MOTORS

Putting a solid rocket motor through DHMR requires heating the entire motor to thermo-equilibrium at the desired temperature and holding at that temperature for a specified time. For large motors, the temperature ramp-up and ramp-down process causes the bore surface and the insulation surface to remain at the high temperatures for much longer than the rest of the propellant in order to achieve thermo-equilibrium. These surfaces experience significant loading during ignition and firing of the SRM. Solid propellant is a viscoelastic material, meaning when it is stressed or damaged from a process like DHMR some of the material properties are permanently degraded unlike elastic materials. These changes in the stress strain curve are shown in Figure 2.

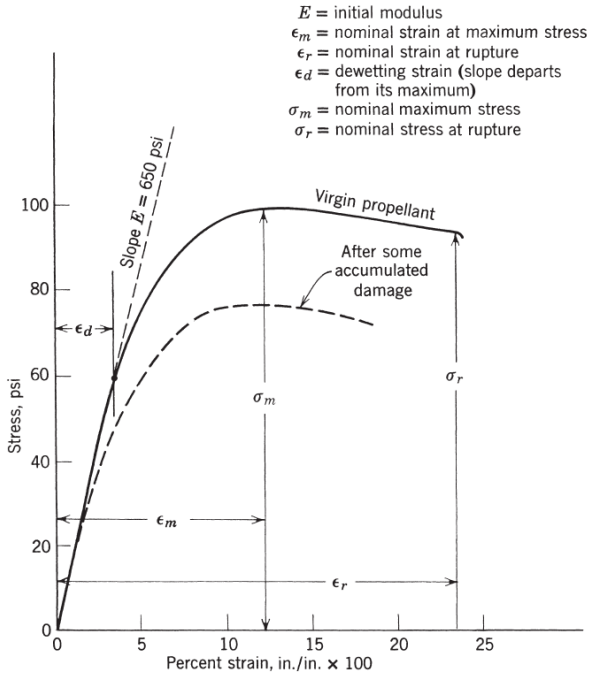

Figure 2: Stress and strain curve of solid propellant. Solid propellant is a viscoelastic material meaning once the propellant is damaged the properties degrade [7].

DHMR can be effective in bioreducing a motor but it may, in some cases, lead to potentially significant and detrimental motor material capability reduction due to the heating process effects on the propellant grain. The effect of heating is similar to the effect that aging has on the propellant The potential for decreased reliability of the motor due to damage from DHMR creates a need for a new strategy to achieve maximum probability of mission success with SRMs.

VHP is another common way to bioreduce components for planetary protection. During this process 30% hydrogen peroxide is used as a sterilant gas. The gas is pumped into an enclosed volume and effectively sterilizes all exposed surfaces. VHP has been widely used for

routine decontamination of enclosed environments and surfaces. There are components of the SRM that are not compatible with VHP. This requires awareness during material and component selection to ensure compatibility with VHP or the ability to isolate the components that are not compatible.

For missions, such as the Europa Lander, where heat sterilization or VHP of all components will lead to reduction in the reliability of the mission or are not comprehensive enough, a different strategy must be used. The proposed planetary protection strategy for the Europa Lander mission is shown in Figure 3. The strategy consists of multiple bioreduction techniques, including DHMR and VHP. During manufacturing of the motor the case, liner, and insulation will go through heat treatment or curing cycles that are similar to DHMR. The separate component systems will undergo DHMR prior to assembly on the motor. The propellant is not planned to undergo DHMR due to the effect on material capability. During operation of the SRM the propellant is complexly combusted at a temperature exceed 3000 °C which will eliminate all extant microorganisms within the propellant. The system will be equipped with a failsafe device that will ensure ignition of the SRM prior to contact with the Europan surface. Once the motor is fully manufactured a nozzle bio cover will be installed to create two separate volumes, external and internal. The motor and components will then be integrated together with the lander, and the upper volume of the lander will go through VHP. The nozzle materials may be damaged by exposure to VHP and are planned to be sealed off. Further, exposure to the propellant combustion gases, which are above 3000 °C, will bioreduce the internal motor components.

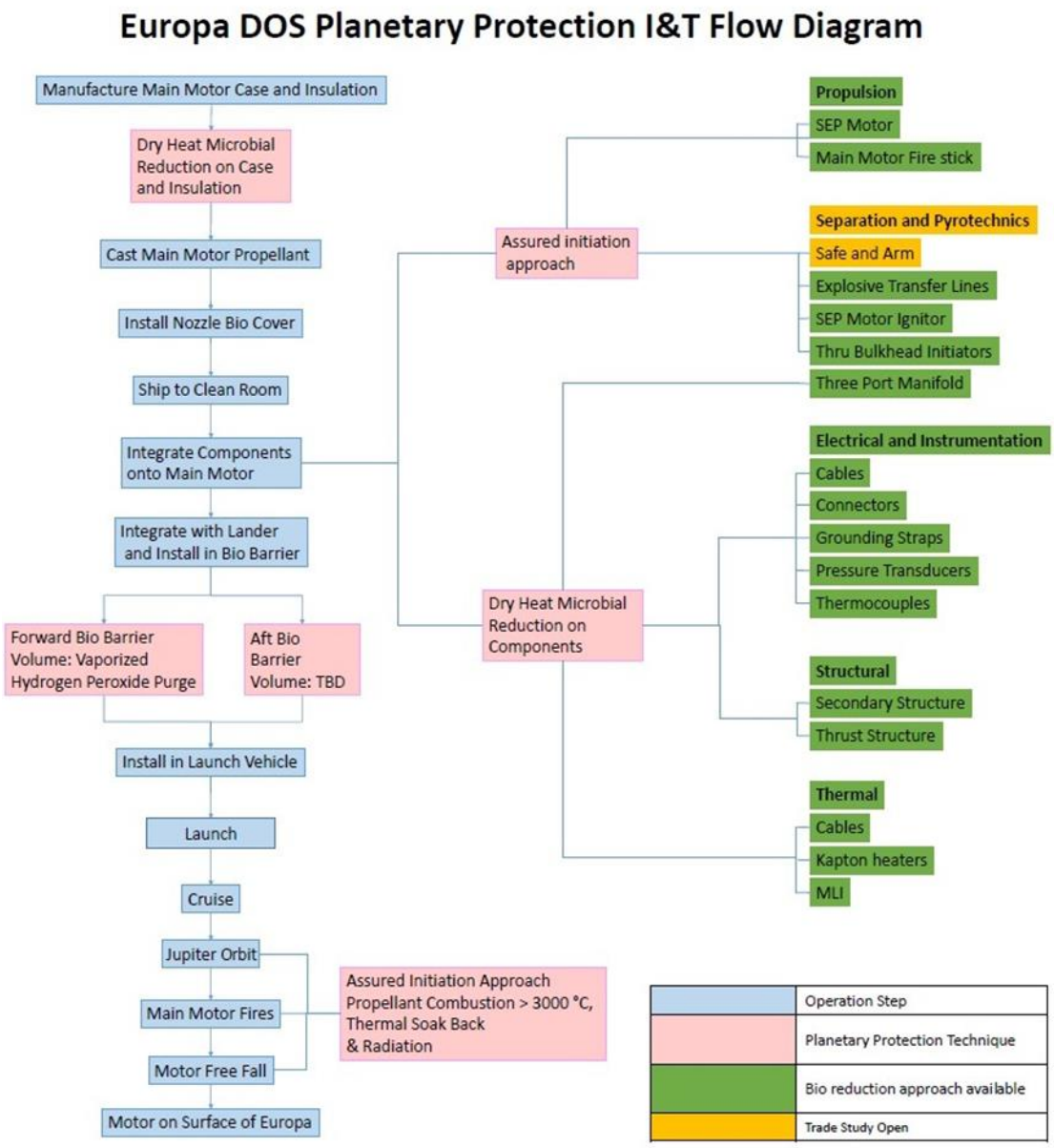

Figure 3: Europa Lander DOS planetary protection strategy.

The planetary protection strategy is progressing with the Europa Lander program, and will continue to be investigated and matured. Research into the mated motor surfaces and the bioreduction capabilities of the propellant, liner, and insulation materials has been conducted and is discussed in following sections.

INVESTIGATION INTO ANTIMICROBIAL PROPERTIES OF BOND-LINE MATERIALS

The presence and survival of microbes encapsulated in the polymer matrix of the bond-line materials in the solid rocket motor poses a risk of forward biological contamination [8, 9]. A significant portion of the bond-line undergoes in-place vulcanization of the insulation materials within the rocket motor case. This vulcanization process typically exceeds the DHMR requirements for time at temperature. However certain locations within an SRM, often where the insulation is thick or a controlled insulation shape is required, the insulation is vulcanized off

motor and secondarily into the case with adhesives. For these locations then, recontamination of the external surface is possible and it is desirable to investigate and potentially benefit from any antimicrobial properties of bond-line materials, namely the bond-line primer and adhesive

The insulation bond-line of a solid rocket motor comprises a combination of a primer (Chemlok® 205) and one of the adhesives (Chemlok® 234X, 2332, 6250, or 6450) to adhere the titanium case to the insulation. Consequently, a combination of an overlay assay and a Kirby Bauer test were utilized to perform a preliminary screening of the antimicrobial properties of bond-line adhesives.

To assess the microbiome of an inert solid rocket motor propellant sample, the microbial culture was obtained via submersion of a bulk material sample of 3.97 cm^3 (2.50 cm X 2.50 cm X 0.635 cm) in 10.0 mL Trypticase Soy Broth (TSB; BD BBL™). The bulk propellant was cut with a guillotine and blade (sterilized prior to use). To best reflect the microbiome entrained in the propellant, samples were taken from the interior of the bulk propellant. The inoculum was incubated at 32 °C for 5 days.

Trypticase Soy Agar plates (TSA; BD BBL™) were streaked in accordance with the methodology described by the Clinical and Laboratory Standards Institute [10]. TSA plates, as the specified media for NASA bioassays [11], were used in lieu of Muller-Hinton agar plates. A sterile, non-toxic cotton swab (Puritan) was dipped into the inoculum suspension, rotated several times, and pressed against the wall of the tube to expel excess inoculum. The swab was streaked over the surface of the TSA plate to inoculate the plate. In two subsequent streaks, the plate was rotated 60 degrees, and the swab spread over the entire surface. The process was repeated for 5 TSA plates per adhesive, a total of 20 plates.

After the inoculum dried, Chemlok® 205 (LORD, Cary, NC), was applied in accordance with the manufacturer's instructions to 1/3 of each plate using a sterile swab (Puritan) [12]. The primer was applied uniformly to a dry thickness of approximately 10 microns and allowed to cure at ambient temperature for 60 minutes. Subsequently, Chemlok® 2332 (LORD, Cary, NC) was uniformly applied over the primer to a dry thickness of approximately 15 microns and allowed to cure for 60 minutes [13]. Following the allotted cure time, the plates were sealed, inverted, and incubated at 32 °C, in accordance with the NASA Bioassay Handbook NASA-HDBK-6022 instructions for optimal growth [11, 14, 15]. A positive control in this experiment was established by plating an undiluted inoculum. A negative control was established by plating sterile TSB. The process was repeated for each of the adhesives (Chemlok® 234X, 2332, 6250, and 6450).

At the 24 hour observation, a zone of inhibition was apparent on each of the plates as seen in Figure 4. The magnitude of the zone of inhibition differed among the Chemlok® adhesives (Table 1).

Table 1: Average values for measured zones of inhibition after 24-hour incubation; zones were measured from the edge of the Chemlok® adhesive to the first observed colonies.

	Chemlok 234X	Chemlok 6250	Chemlok 6450	Chemlok 2332
Zone of Inhibition (mm)	67.0	48.0	90.0	46.0

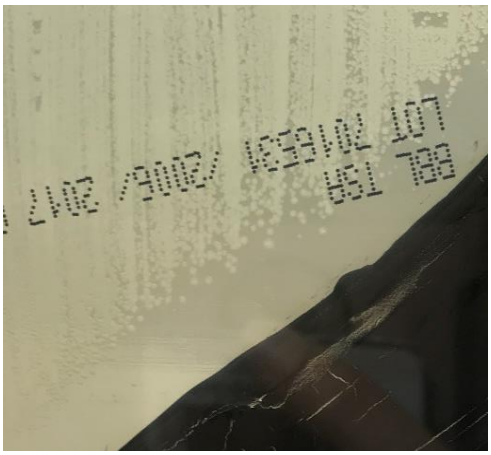

Figure 4: Zone of inhibition following exposure to Chemlok® 6450.

The zones of inhibition present in the preliminary screenings indicate biostatic or biocidal activity in the Chemlok® formulations. This result is promising in that biocidal activity that may be caused by the adhesive-primer can add to the bio-reduction in these areas and reduce risk of forward biological contamination by the SRM.

SOLID ROCKET MOTOR MATED-CASE STUDIES

In solid rocket motors, the case is mated to the nozzle with a ring of fasteners and washers. These mated areas of SRMs (joints) represent a challenge to integrated sterilization outside of cleanrooms. The challenges occur due to the complex geometry in bolts, screws, and washers. Also, materials within the mated areas may be incompatible the VHP process that may be used in motors which do not undergo DHMR [16, 17]. It is hypothesized that bolt heads with aerospace quality surface finish may contain irregularities large enough that they might become safe havens for microbial organisms. These cells, which could not survive under pressure, could survive in these pockets created by surface roughness, sealed off between a mated surface and grease. Multiple French press experiments have been done throughout history in the microbiological sciences to understand pressure resistance of vegetative cells and spores [18]. A mechanical cell lysis analysis was the chosen approach to better understand the variables at play in a mock assembly process.

A fastener torque tension analyzer machine was used in force array intervals of 5 kips held for 20 seconds on each fastener. The torque tension analyzer settings for the 5 runs are summarized in Table 2. Fasteners and washers were autoclaved prior to use and fasteners were inoculated with 15 µL of a native (A) *Bacillus sp.* overnight TSB stock on the previously marked area. Washers were swabbed with Royco® 43 SAE AMS-G-4343 grease prior to torque tension testing. Fastener head surfaces were aseptically swabbed, and the swab was submerged in 4 mL of dihydrogen phosphate buffered distilled water at pH 7.2+0.1 per the NASA Handbook for the Microbial Examination of Space Hardware NASA-HDBK-6022 specifications [15]. The same was done for the washer sides that made contact with the fastener surface. All swabs used were sterile.

Table 2: Load amount exerted on fasteners labeled by numbers.

Fastener #	Force Array (kip)	Time (s)	Torque (ft·lb)
1	15.0	20	109.2
2	10.6	20	85.2
3	5.0	20	40.9
4	1.0	20	11.8
5	0	20	0

Native sporulating bacilli were isolated from a propellant mix/cast laboratory surface areas via surface swabbing and buffer submersion. Buffer samples underwent heat-shock treatment per NASA-HDBK-6022 specifications [11, 15]. Spore content left in the tube was spread plated (1mL) on TSA and incubated for 24 hours in 32°C. The plates were shipped and commercially sequenced using the 16S Ribosomal RNA and compared against existing NCBI GenBank sequences to assure different native species are being used.

Conical tubes containing the torque tension sample swabs and buffer were vortexed and the entire buffer content was poured in 10 mL TSB and incubated at 32 °C for 24 hours. After incubation 10 µL from each tube were used to find the CFU/mL from a 10^{-12} total dilution factor after 24 hours of incubation at 32°C. A previous experiment on the same fasteners using the agar pouring techniques showed a slow rate of growth in bacilli making it inadequate for colony counting strategies under 72 hours.

After 24 hours of growth, for fastener/washer #5 there were too many colonies to account for. For the rest of the fasteners/washers the combined growth in CFU/mL puts fastener #4 with the highest amount of CFU/mL and lowest force array/torque exerted. The fastener labelled #1 had the lowest CFU/mL and was the fastener/washer combination to have the highest torque applied. The results are shown in Figure 5. A higher number of torque levels might be able to help infer a correlation between the increase in CFU/mL and a decrease in torque application with a specific bioreduction number. Higher torque levels might be needed to get a reasonable bioreduction. Notably, spore preparations and spore bioreduction calculations should be the focus of future experiments since spore reductions are of interest to the Office of Planetary Protection [20].

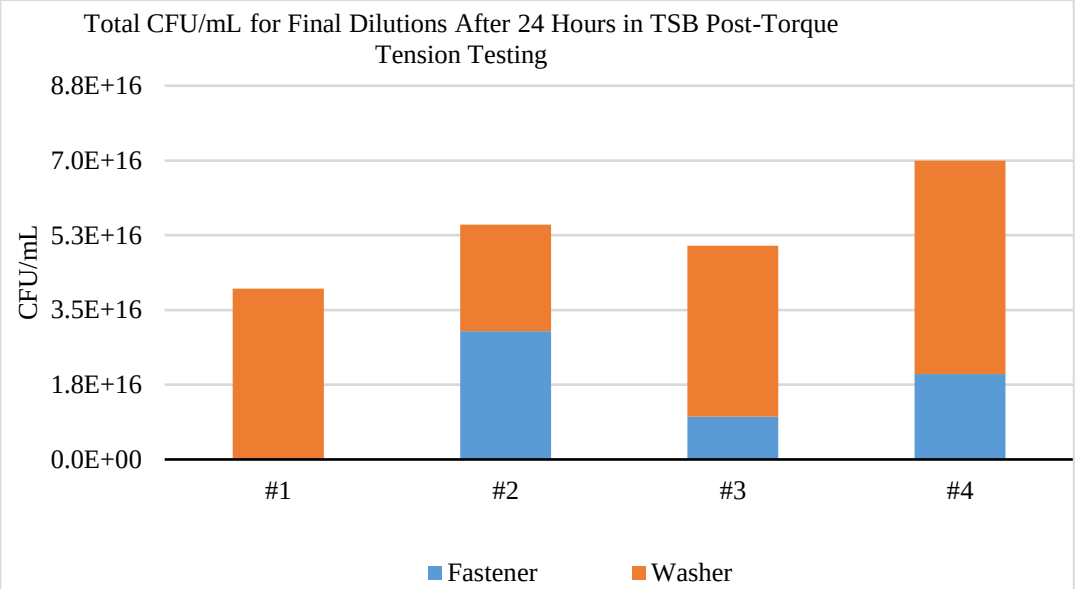

Figure 5: Combined CFU/mL from washers and fasteners samples after torque application.

Four common aerospace grease types Royco® 43 SAE AMS-G-4343, Molykote™ G-n Metal Assembly Paste, Krytox™ PFPE, and Braycote® 803RP were tested using the Kirby Bauer method against the three different native (A, B, and C) bacilli. AMS-G-4343 was heated to a boiling point of 120 °C to be used in dilutions where the grease comprised 50% of total weight to find the 10^{-12} total dilution factor in 24 deep well plates with bacilli A from a 24-hour TSB stock solution.

For the Kirby Bauer tests done with Royco® 43 SAE AMS-G-4343, Molykote™ G-n Metal Assembly Paste, Krytox™ PFPE, and Braycote® 803RP none of the greases showed zones of inhibition after 24, 48 and 72 hours, indicating no effect of the grease on the studied (A, B, and C) microorganisms, as shown in Figure 6. From the dilutions done in bacilli A, the final dilution showed a decrease in CFU/mL compared to the control after 24 hours. The average CFU/mL from the replicates at 10^{-12} was $3.33x10^{10}$, which will only occur if grease components are perturbed by heat, was compared to $1.6x10^{12}$ in the control. Further studies will consider the application of pressure with possible effects on grease on motor assembly pre and post operation.

Figure 6: Kirby Bauer tests done on Royco® 43 SAE AMS-G-4343 with three different species of bacilli showing no zones of inhibition.

The surface inspection was performed by evaluating the surface area between four half-inch fasteners (NAS673 Ti6Al-4V) commonly used in aerospace applications. The inspection was performed with a Keyence VR-3200 which was used to inspect rubber molds created from the fasteners. The system utilized high magnification, 80X zoom, and superfine type scanning of

horizontal and vertical lines every 90° through a 13206.025 µm x 17,033.184 µm marked two-dimensional surface on the rubber mold.

Generally, the Sa (Arithmetical Mean Height) or the difference in height of each point compared to the arithmetical mean of the surface is used to evaluate surface roughness whereas the Str (Texture Aspect Ratio) is a measure of uniformity of the surface texture and uses 1.0 to represent a completely isotropic surface. The final Str value was not in isotropic equilibrium indicating some qualitative and quantitative unevenness of the surface shown from this experiment. From the 224.941 mm^2 scanned two-dimensional surface area a maximum overall height of peaks of 20.150 µm and a height difference representative of distance between lowest valleys and highest peaks of a surface were found with an average of 1.289 µm. The average two-dimensional height differences were 186.911 µm by 3.398 µm (this is the size of a potential "safe haven" for microorganisms). To put this in perspective *Bacillus subtilis* is typically 1 x 6 µm [19]. These measurements are summarized in Table 3.

Table 3: Measurements of four NAS673 Ti6Al-4V fastener head surfaces from a marked area via Keyence VR-3200.

Average Distance Between Lowest Valleys and Highest Peaks	Maximum Overall Height	Average Height Difference (length)	Height Max. (length)	Average Height Difference (width)	Height Max. (width)	Standard Deviation
1.289µm	20.150µm	186.911µm	531.130µm	3.398µm	8.168µm	0.000 µm

SUMMARY AND CONCLUSIONS

Exploratory missions that use solid rocket motors face challenges with traditional planetary protection techniques. Missions like the Europa Lander that use an SRM may choose to forgo traditional techniques of DHMR and VHP where these techniques reduce the overall reliability of the SRM. These missions must continue to meet the stringent requirements posed on class III, IV, and V missions and strategies involving multiple techniques must be used. The strategy proposed uses DHMR on non-sensitive components prior to full integration, a two volume approach with the SRM to allow the internal surfaces to undergo a different technique than the external surfaces, and VHP once full integration has occurred and sensitive components are protected.

Experiments have been conducted to look at the bioreduction capabilities of motor components as well as areas that may be difficult for some bioreduction techniques to meet. The current strategy allows the Europa Lander to meet the stringent planetary protection requirements and does not impact the reliability of the motor or jeopardize mission success.

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