

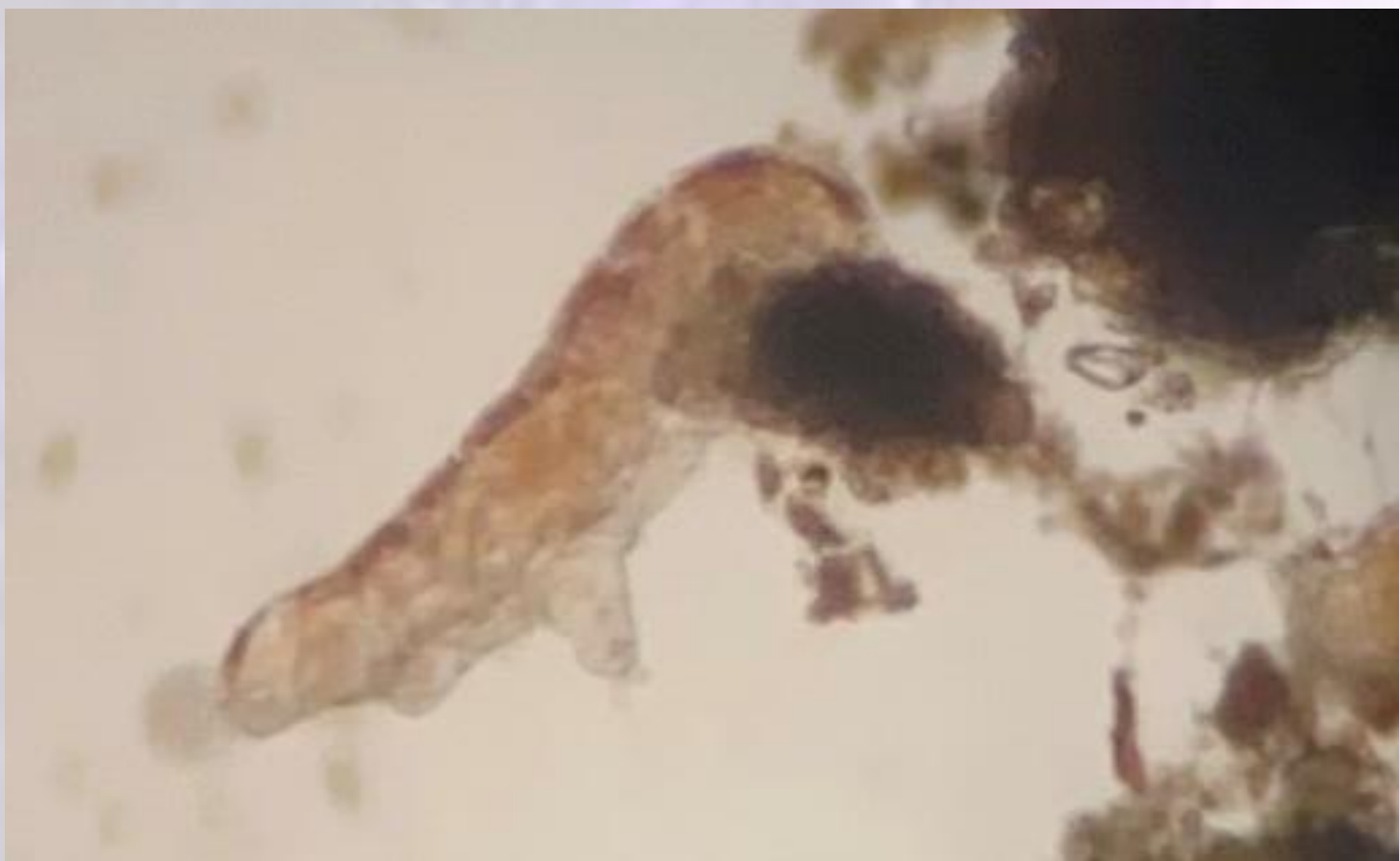
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Background and Significance

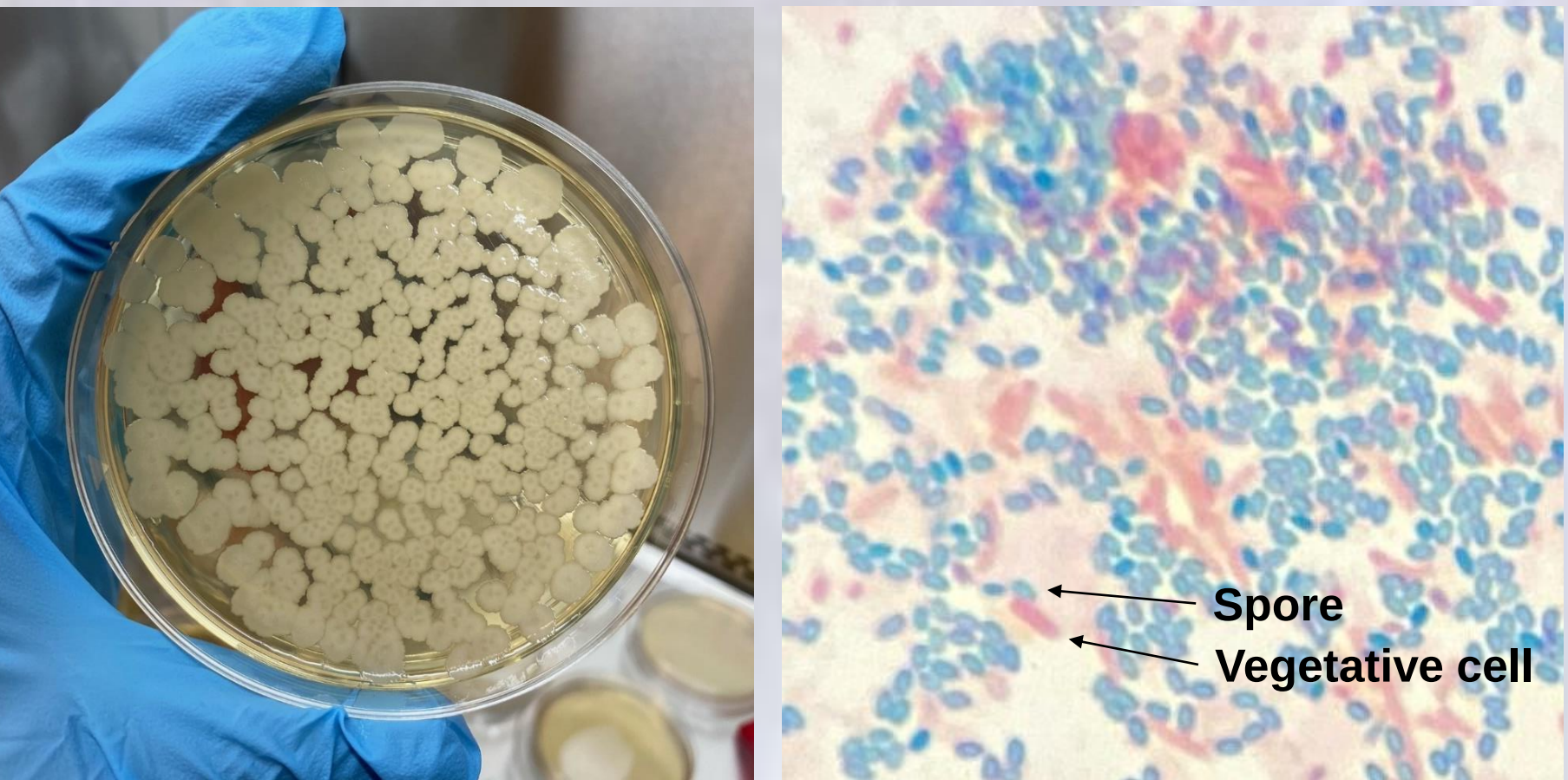
Planetary Protection is defined by NASA as the practice of protecting solar system bodies from contamination by Earth life and protecting Earth from possible life forms that may be returned from other solar system bodies. It is particularly relevant for missions focused on life detection. Simplified, **PP is important for three main reasons:**

- 1) To maintain integrity of science conducted, hardware functionality, and purity of samples collected
- 2) Ethically, to preserve pristine environments and avoid contamination/seeding with Earth microbes
- 3) To protect Earth from possible microbes or bioactive molecules returning in samples or on spacecraft



A tardigrade at 100x magnification

PP requirements are defined by NASA's Planetary Protection Officer (PPO) and described in NPR 8715.24¹. PP is a relatively new and expanding field at MSFC. PP specialists help navigate requirements and communicate with other NASA centers as well as vendors to ensure compliance. This can include physical implementation during build or assembly and test operations, as well as insight and oversight to help vendors ensure proper implementation.



Bacillus atrophaeus colonies on a petri dish (left) and as imaged through a light microscope at 1000x magnification with spores stained in blue and vegetative cells in pink (right).

In recent years, MSFC has also expanded to include PP research which is primarily concerned with identifying microbial threats and developing novel methods to neutralize those threats. These research goals are summarized in three main areas:

- 1) Enumeration of bioburden from nonmetallic spacecraft materials
- 2) Bioburden reduction credit through manufacturing processes
- 3) Space environmental effects on microbial survival

1. Bioburden of Nonmetallic Spacecraft Materials

The greatest threat to achieving PP requirements is the bioburden encapsulated within nonmetallic spacecraft materials. Materials comprising spacecraft hardware must be "bioburden reduced" in conformance with NASA's mission requirements. Typically to meet PP requirements, such materials must undergo bakeouts at set temperatures for a specified time course in order to reduce the bioburden to an adequate degree. However, some missions are limited by the use of these bakeout methods as they can be harsh and impact material integrity. If materials cannot undergo bioburden reduction, assumed bioburden "specification values" are applied by NASA which can threaten the project's ability to comply with or meet the PP requirements. Therefore, it is critical to understand the limitations of a mission and/or materials and empirically assess material bioburden where possible.

NASA describes encapsulated bioburden specification values:

- 30 spores/cm³ of nonelectronic materials
- 130 spores/cm³ of mixed nonmetallic assemblies
- 150 spores/cm³ of electronic piece parts

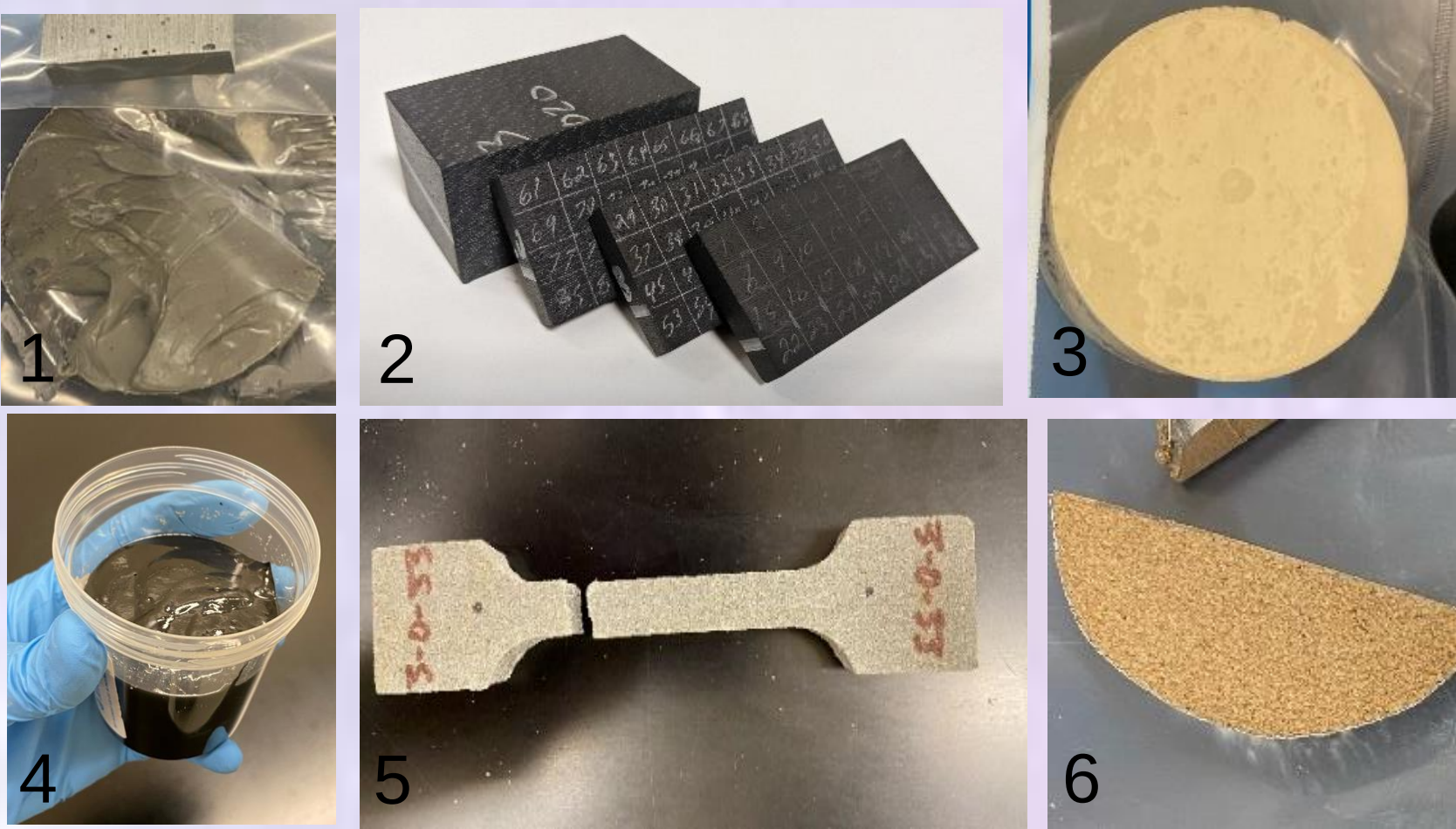
These specification values are:

- **Conservative** (i.e. high end estimates)
- May represent an order of magnitude greater abundance than true numbers
- May be overestimated because many materials contain toxic substances

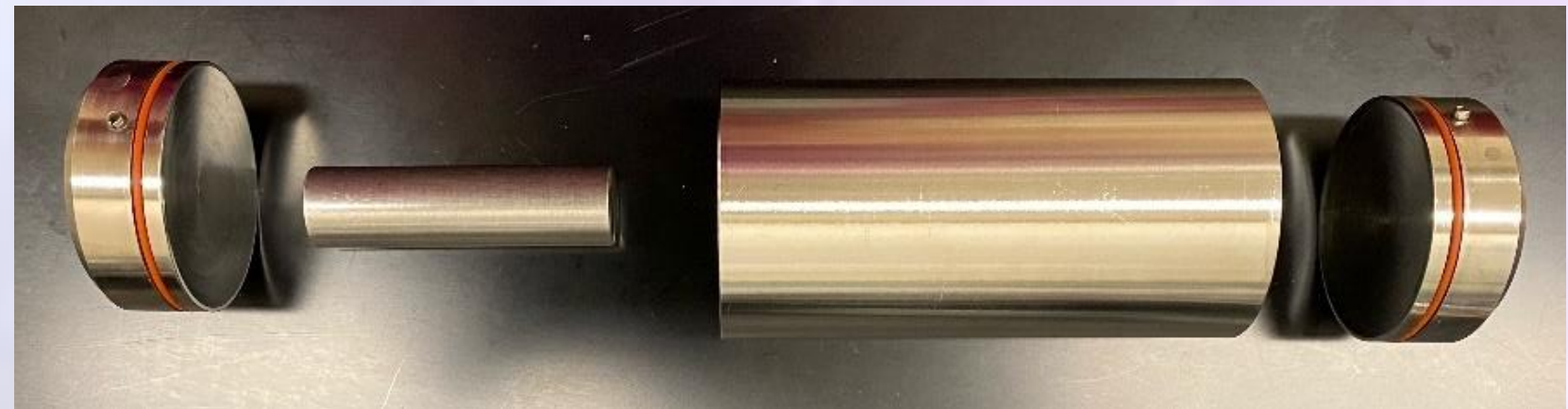
To potentially prevent the use of NASA specification values, the PP lab at MSFC has a SPEX SamplePrep 6875 freezer mill which freezes material samples to cryogenic temperatures with liquid nitrogen, then pulverizes the material into a powder. Once in powdered form, the material can be assessed for bioburden, providing a baseline order of magnitude for the material.

With funding from an ER62 Tech Excellence Award, we assessed the total bioburden associated with 14 different nonmetallic materials, consisting of six categories:

- 1) Adhesives
- 2) Composites
- 3) Insulations
- 4) Liners
- 5) Inert propellants
- 6) Thermal protection systems



Example materials of 1) adhesives, 2) composites, 3) insulation, 4) liner, 5) inert propellant, and 6) thermal protection systems.



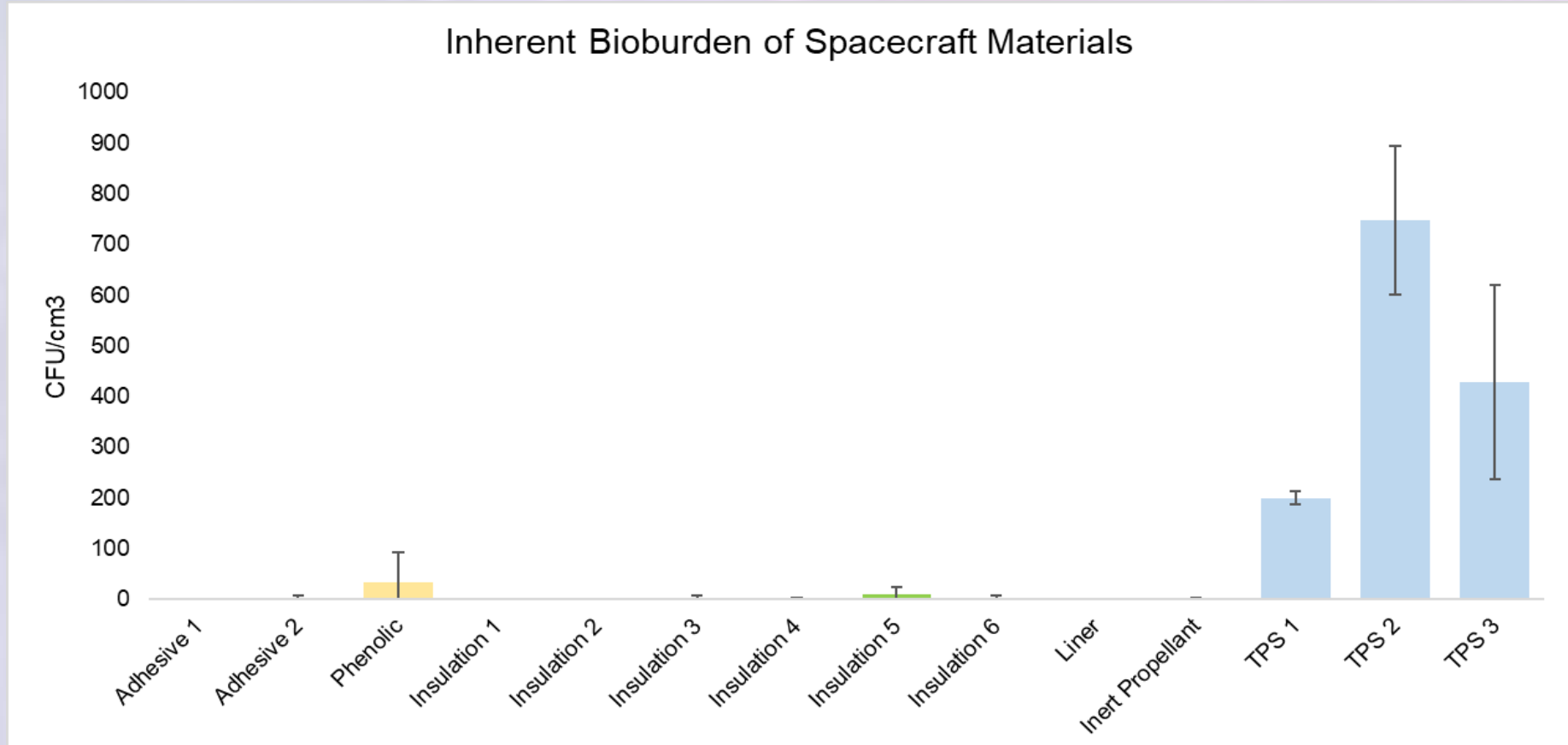
Exploded view of a SPEX SamplePrep cryovial

We developed a protocol based on Schubert *et al.*, 2016². This procedure is a living document and can be updated over time and used for many materials/projects to answer specific questions for certain missions.



Example of experimental flow for bioburden assessment of nonmetallic materials.

We found that the majority of materials tested demonstrated smaller total bioburden than the NASA specification values would estimate. Therefore, applying empirically-determined estimates of bioburden can lower total accountable bioburden for certain materials, reducing the risk associated with materials limited in bioburden reduction.



Total bioburden associated with nonmetallic spacecraft materials

2. Manufacturing and Bioburden Reduction

When material properties present limitations to achieving bioburden reduction, one contingency is to take "credit" for manufacturing processes that may reduce the bioburden to a satisfactory degree. One such manufacturing process is the cure cycles that rubber insulation must undergo. While these cycles typically reach high temperatures for extended periods of time, the cycles vary depending on the materials and formulations used.

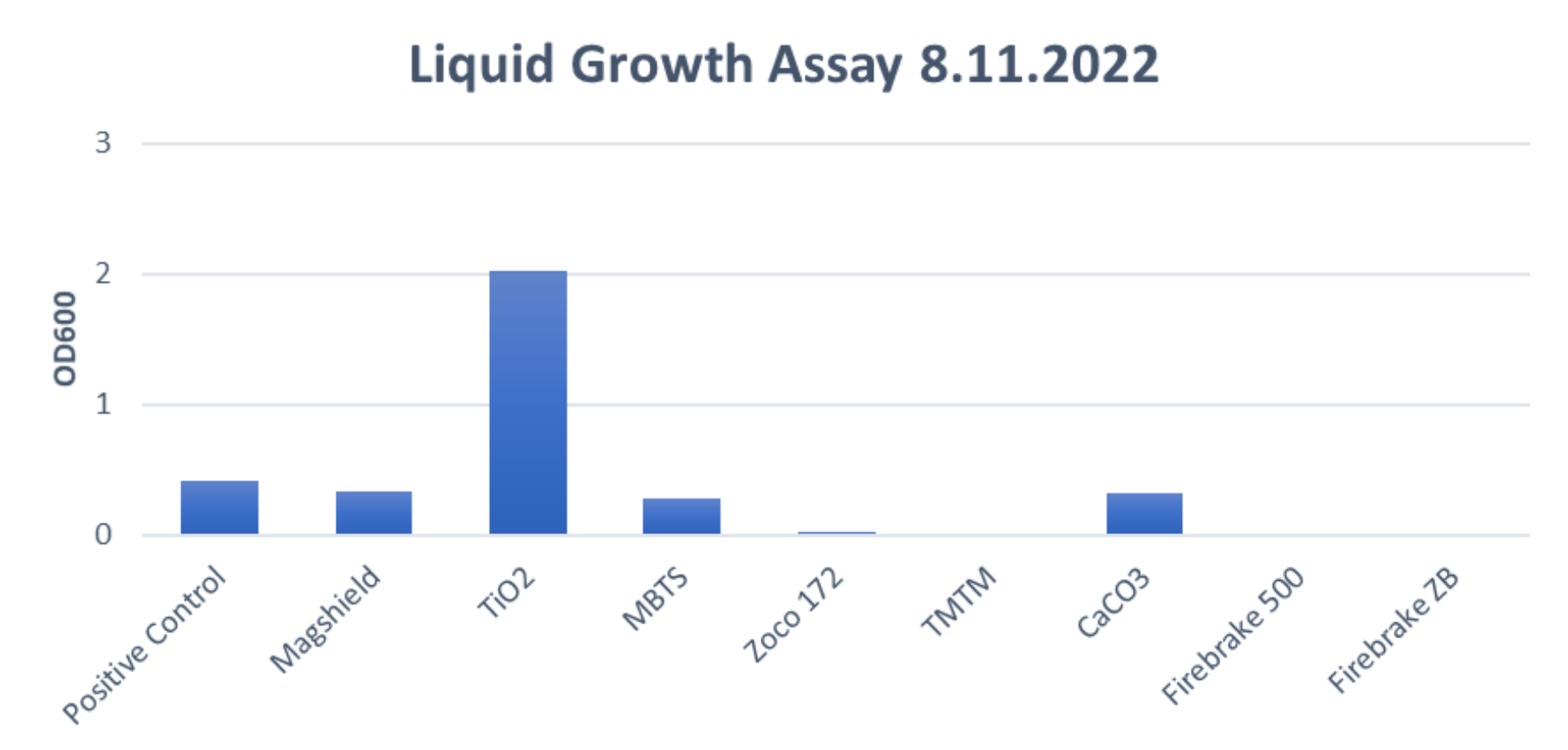
With funding from a NASA ROSES grant awarded to collaborators at Auburn University, we wished to empirically assess if a typical insulation cure cycle resulted in adequate bioburden reduction. To achieve this, we intentionally inoculated the interior three layers of green rubber with 10⁹ CFU/ml *B. atrophaeus* spores which were allowed to dry. We then subjected samples to a typical cure with thermocouples in place.



Green rubber insulation inoculated with 10⁹ CFU/ml *B. atrophaeus* spores (top). Insulation panels post-cure (bottom).

From the thermal profile, we found that all insulation layouts endured approximately 9 hours at ~275°F (~135°C). When compared with the required time and temperature durations described in the European Space Agency's guidelines³ (by which NASA also adheres), this cure cycle well exceeds the required time of 100 minutes to reduce encapsulated bioburden by 2- or 3- orders of magnitude.

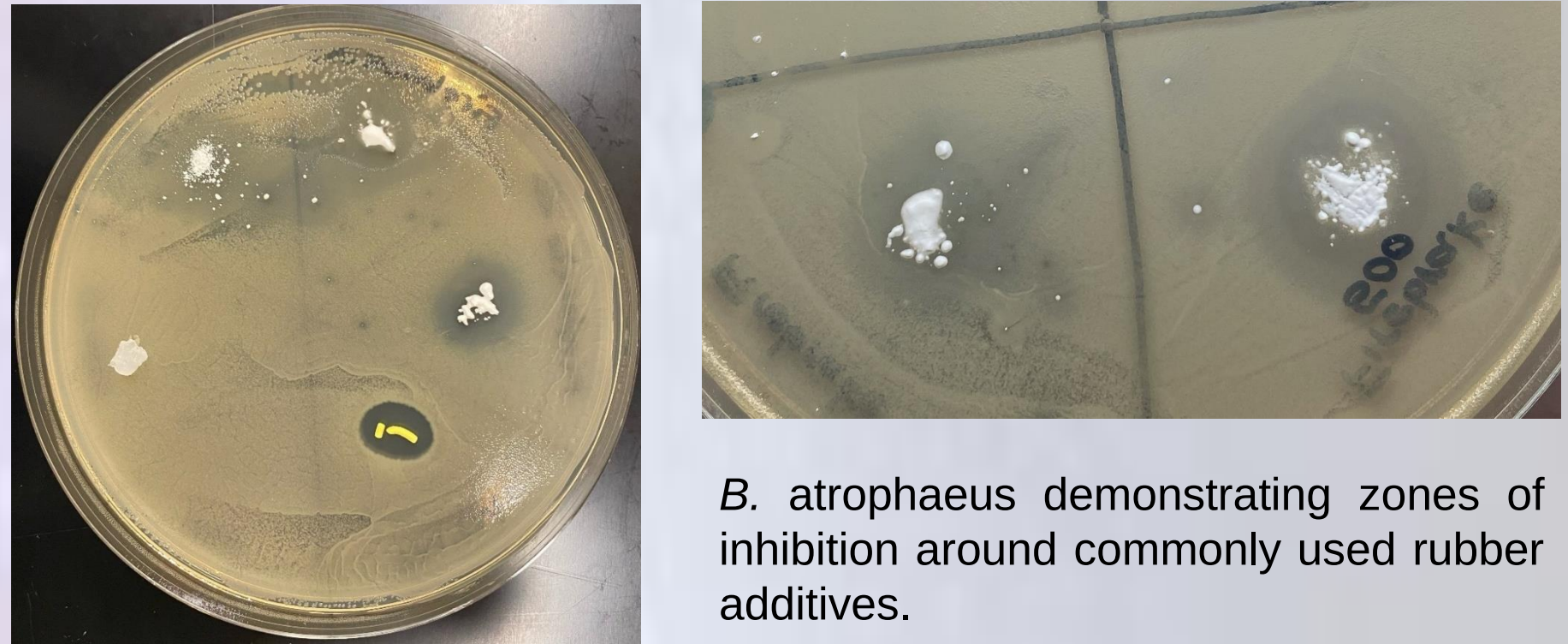
To empirically assess if the cures were sufficient to reduce the inoculated spores to undetectable levels, we will employ our destructive cryogenic analysis and assay for *B. atrophaeus* survivors. These analyses are currently underway.



Growth inhibition demonstrated in liquid culture when *B. atrophaeus* is grown with commonly used insulation additives.

An additional method of taking credit for manufacturing processes as bioburden reduction is to utilize materials or chemicals that are inherently antimicrobial. For example, many additives used in formulations of insulation are known to be toxic and may have effects on microbial survival, thereby reducing the microbial load encapsulated within the material.

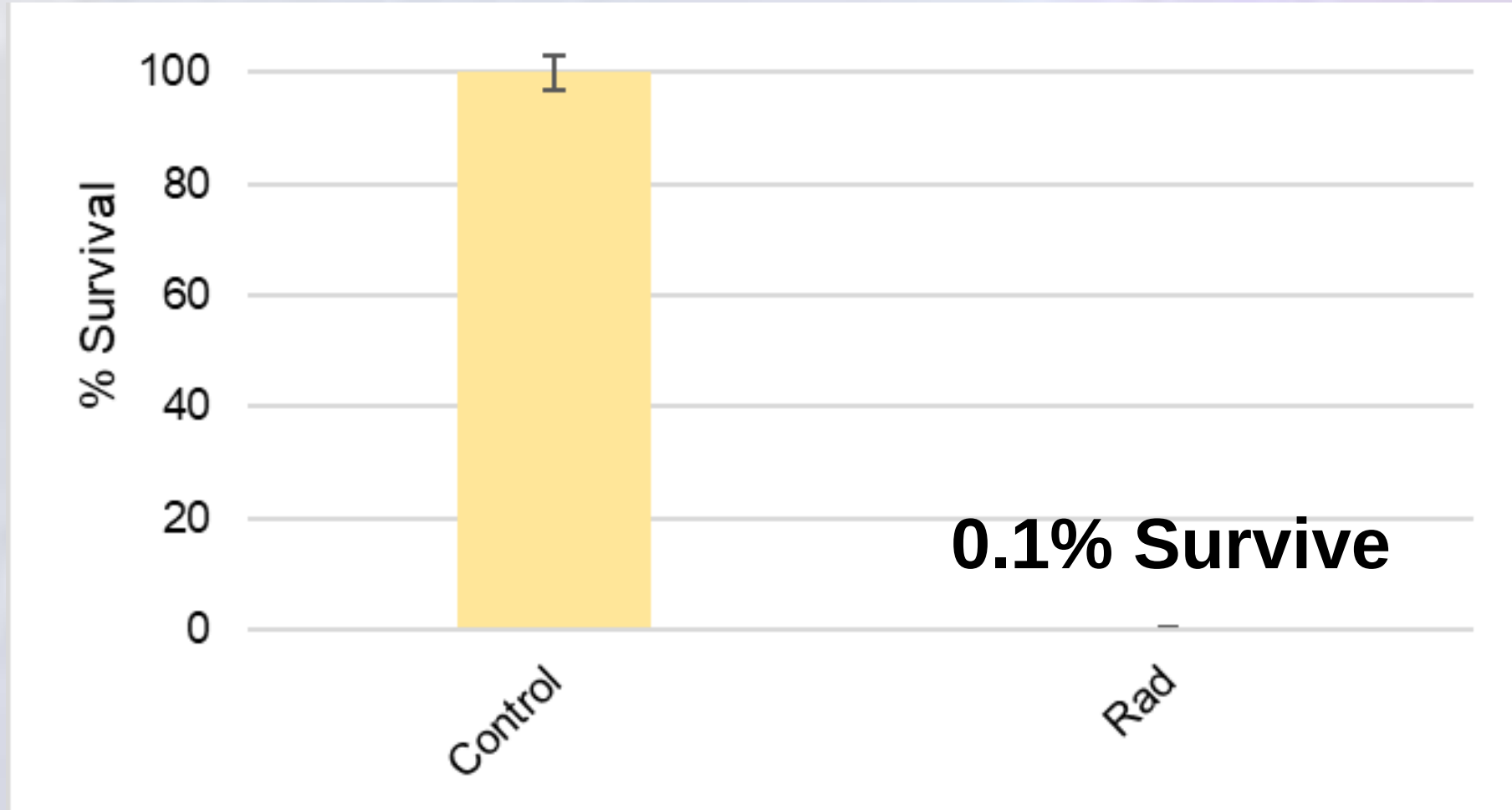
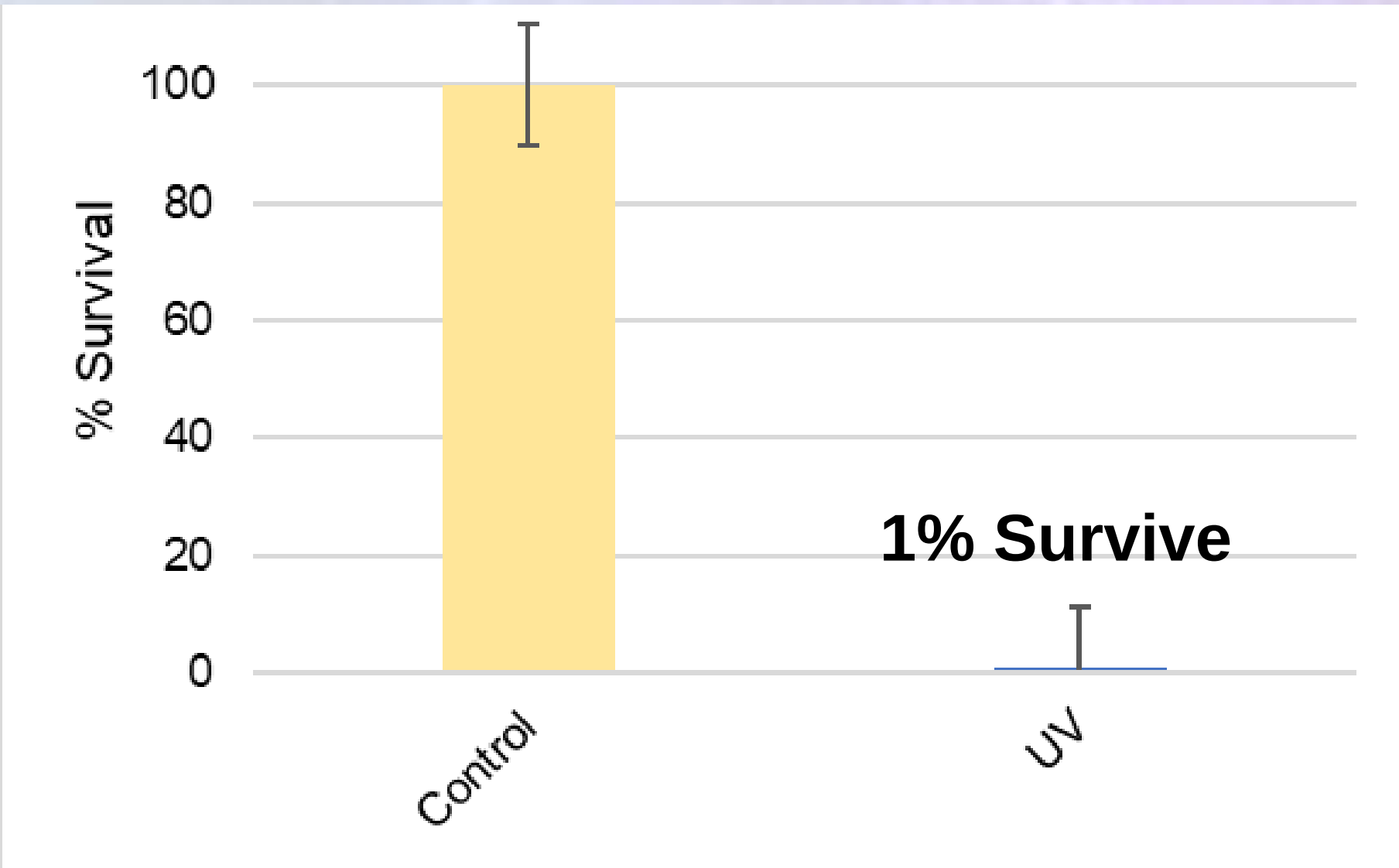
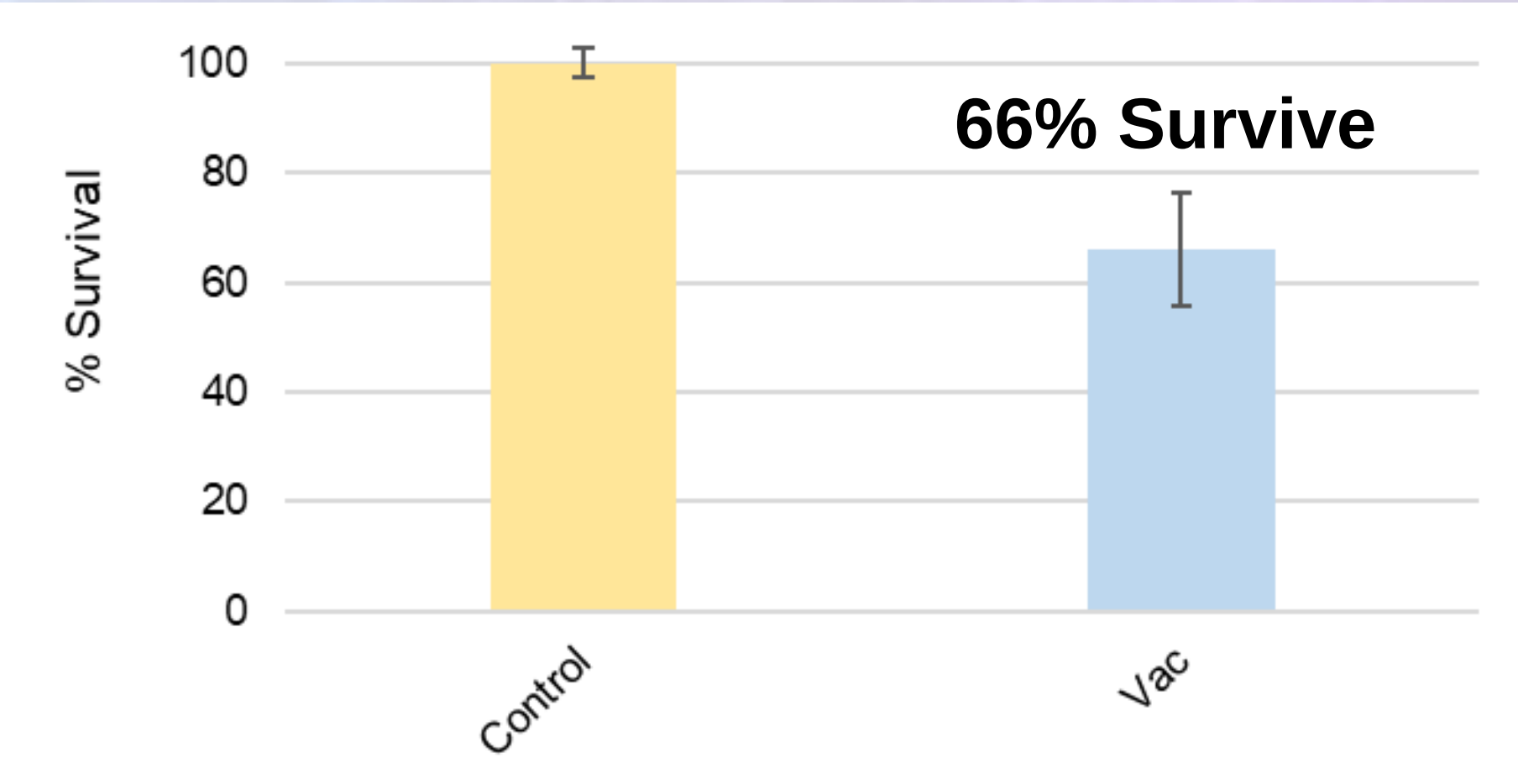
To assess the antimicrobial effects of commonly used insulation additives, we performed a spot-on-lawn assay with *B. atrophaeus*. We saw that several additives prevented the growth of the bacteria, supporting the possible use of these chemicals in insulations mixes where it could be beneficial both for material properties and bioburden control.



B. atrophaeus demonstrating zones of inhibition around commonly used rubber additives.

3. Space Environment Effects on Microbe Survival

Finally, the space environment consists of many harsh variables, including vacuum, ultraviolet (UV) radiation, ionizing radiation, and temperature extremes. Because of these inhospitable conditions, the space environment represents a potential source of bioburden reduction. However, while it is clear that these conditions are harsh, the extent of lethality to microbial cells subjected to this environment is unknown. As a result, we have performed some initial characterization microbial viability in response to vacuum, UV radiation, and ionizing radiation.



Percent survival of *B. atrophaeus* exposed to space environments.

Based on these findings, the space environmental variables of vacuum, UV, and ionizing radiation drop the viability of *B. atrophaeus* spores to 66%, 1%, and 0.1% respectively. However, while these are significant decreases, there are still survivors detected. Future work will include an additive exposure to better understand the effects of the combined environment on survival.

Conclusions

These findings describe the current work ongoing within MSFC's PP lab. The results here provide evidence that there are likely far fewer contaminating microbes within nonmetallic materials than assumed by NASA's specifications. Furthermore, this work has demonstrated the usefulness of relying upon both manufacturing processes as well as the space environment as bioburden reduction techniques, which may reduce cost and time.

Given NASA's interest in Mars as well as other destinations in the outer solar system, PP is becoming more important in mission design and execution. Thus, MSFC is expanding PP while also contributing to the field by providing the data described in this poster. Furthermore, we are working to secure additional funding to continue many of the experiments described here, while also looking to expand and build PP by way of collaborations both inside and outside of MSFC and NASA at large.

¹ NASA Procedural Requirement 8715.24: Planetary Protection Provisions for Robotic Extraterrestrial Missions
² Schubert, W.W., Newlin, L., Chung, S.Y., Ellyin, R. Assessment of bioburden encapsulated in bulk materials, *Advances in Space Research*, Volume 57, Issue 9, 2016, Pages 2027-2036, ISSN 0273-1177, <https://doi.org/10.1016/j.asr.2016.02.012>.
³ ECSS-Q-ST-70-57C-DFR1