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Assessment of bioburden encapsulated in bulk materials

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Abstract

The National Aeronautics and Space Administration (NASA) imposes bioburden limitations on all spacecraft destined for solar system bodies that might harbor evidence of extant or extinct life. The subset of microorganisms trapped within solid materials during manufacture and assembly is referred to as encapsulated bioburden. In the absence of spacecraft-specific data, NASA relies on specification values to estimate total spacecraft encapsulated bioburden, typically 30 endospores/cm^3 or 300 viable cells/cm^3 in non-electronic materials. Specification values for endospores have been established conservatively, and represent no less than an order of magnitude greater abundance than that derived from empirical assessments of actual spacecraft materials. The goal of this study was to generate data germane to determining whether revised bulk encapsulated material values (lower than those estimated by historical specifications) tailored specifically to the materials designated in modern-day spacecraft design could be used, on a case-by-case basis, to comply with planetary protection requirements.

Organic materials having distinctly different chemical properties and configurations were selected. This required more than one experimental and analytical approach. Filtration was employed for liquid electrolytes, lubricants were suspended in an aqueous solution and solids (wire and epoxy sealant) were cryogenically milled. The final data characteristic for all bioburden estimates was microbial colony formation in rich agar growth medium. To assess survival potential, three non-spore-forming bacterial cell lines were systematically encapsulated in an epoxy matrix, liberated via cryogenic grinding, and cultured. Results suggest that bulk solid materials harbor significantly fewer encapsulated microorganisms than are estimated by specification values. Lithium-ion battery electrolyte reagents housed fewer than 1 CFU/cm^3. Results also demonstrated that non-spore-forming microorganisms are capable of surviving encapsulation within, and liberation from, epoxy solids. It must be noted, however, that all purposely spiked experimental solids, resulted in very low recovery (1×10^{-3}–1×10^{-5} CFU/cm^3) of viable organisms.

Keywords: Planetary protection; Encapsulated; Bioburden; Spacecraft; Organic materials

1. Introduction

To preserve the scientific integrity of current and future solar system exploration efforts, the National Aeronautics and Space Administration (NASA) imposes cleanliness requirements on all spacecraft intending to land, orbit, or be in the vicinity of any solar system body having the potential to harbor evidence of extant or extinct life. Any mission to a planetary body where water–ice is thought to be present, such as Europa or Enceladus, must also satisfy NASA planetary protection requirements (NPR, specifically 8020.12D) stating that "the probability of inadvertent contamination of an ocean or other liquid water body (by a viable Earth microorganism) shall be less than 1×10^{-4} per mission" (NASA, 2011), Section 5.4.1). The calculation of this probability requires conservative estimation of poorly known parameters, and consideration of the following criteria (at a minimum): (i) total bioburden [the total number of contaminant microorganisms (CM) at

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launch], (ii) survival rate of contaminant microorganisms during cruise, (iii) survival rate of CM in the radiation environment adjacent to the target, (iv) probability of CM encountering/landing on the target (including spacecraft reliability), (v) probability of CM surviving landing/impact on the target, (vi) mechanisms and timescale of transport to the subsurface, and (vii) CM survival and proliferation prior to, during, and after subsurface transfer. Calculating this probability can be challenging given the uncertainty associated with each of these factors. The approach generally taken to solve a multivariable problem such as this is to prioritize those factors that are most influential (and controlling) in the probabilistic calculation outcome and focus technical efforts in those areas first. The authors set out to better understand the first criterion, that is total spacecraft bioburden, paying particular regard to the subset referred to as being encapsulated. Encapsulated bioburden represents the sub-population of microorganisms entrained within solid spacecraft materials during manufacture and assembly.

Studies performed in the 1960s and 1970s document the numerous challenges encountered in estimating the number of microorganisms trapped within solid materials. The recovery rates for endospores were consistently relatively low, typically less than 3% (Angelotti et al., 1968; Gustan and Olsen, 1971). Due to the uncertainty and difficulty in estimating the number of encapsulated organisms in any solid material, NASA standardized initial bioburden densities for encapsulated microorganisms. The agency did not, however, standardize techniques for obtaining encapsulated bioburden densities, as was done for surface bioburden.

In the absence of spacecraft-specific data, NPR 8020.12D (see Appendix D) states that encapsulated bioburden shall be estimated in accordance with the following specification values: 30 spores/cm^3 of non-electronic materials, 130 spores/cm^3 of mixed non-metallic assemblies (inclusive of electronic parts), and 150 spores/cm^3 of electronic piece parts. Intuitively, materials of unknown manufacturing origin/history, or those crafted under uncontrolled, non-cleanroom conditions were initially assigned greater bioburden values. Appendix D of NPR 8020.12D also states that for any given surface or solid volume, there are ten-times as many non-sporulating, vegetative cells present as there are endospores. Hence the encapsulated bioburden specification values of 300 vegetative cells/cm^3 in non-electronic materials, 1300 vegetative cells/cm^3 in mixed non-metallic assemblies (including electronic parts), and 1500 viable cells/cm^3 in electronic piece parts. These specification values for encapsulated bioburden, along with scalability factors to account for non-culturable microorganisms, are used to model and estimate the extent of bioburden dispersion and release in the event of spacecraft impact (assuming total pulverization of that hardware). This brutal impact process would release a great deal of energy, which would be manifested in significant temperature increases in resultant debris. In addition, specification values may be overestimated because the chemical makeup of many spacecraft materials is known or suspected to be toxic (e.g., phenolic resins) to microorganisms. Finally, many solid materials are manufactured and/or cured at temperatures greater than ambient, albeit lower than that required to achieve effective microbial reduction (e.g., cure of two-part epoxies).

Previously, laboratory approaches such as chemical dissolution, grinding with a kitchen blender, and grating soft pliable materials with a cheese grater were tested with varying success (Benardini et al., 2012, 2013). The authors quickly learned that no one technique could be universally applied to all materials. For example, most cured epoxies are insoluble in solvents tolerated by bacterial spores (Stam et al., 2012). Furthermore, while the authors have successfully recovered bacterial endospores from poly (methyl methacrylate) (PMMA) via dissolution in organic solvents (Mohapatra and La Duc, 2012a,b; Stam et al., 2012), vegetative cell membranes are lysed and cell components are inactivated by these harsh chemicals. Grinding with a conventional food blender can effectively reduce the size of solid materials to fine fragments, but extreme care must be taken to avoid overheating due to friction and thus cell death. Other grinding techniques have been employed by other investigators (Gustan and Olsen, 1971), none of which reported significant endospore recovery rates. Citing reported problems with viable microbial recovery from solids, Bauermeister et al. (2014), reported similar low level polymer bioburden recoveries of <0.1–2.5 CFU/cm^3. They also studied direct microscopic observation and molecular approaches. Based on our laboratory's previous success in pulverizing spacecraft solids into fine powders, thereby facilitating the recovery of endospores from solid epoxy, Lucite, silicone elastomer coatings, and electronic parts (e.g., integrated circuit chips, resistors), a cryogenic milling technique was used to grind the solid samples created and analyzed in this study. Upon release via cryogenic grinding, encapsulated cells and spores are released from the solid matrices via physical impact, where the solid material easily fractures at liquid nitrogen temperatures. Once liberated, cells and spores are capable of germinating and proliferating into detectable colonies on rich agar growth medium. While this technique may not be ideal, after having conducted a rather thorough learn-as-you-go survey of available approaches, the authors are confident in advocating cryogenic milling as the best method for viable microorganism detection available at this time. Indeed, cryogenic grinding techniques have facilitated rapid and reproducible sample processing while minimizing the heat stress typically associated with grinding. All of this aside, cryogenic grinding remains a conservative approach, as it is a much gentler process than total spacecraft pulverization due to impact.

The difference in encapsulated bioburden between specification and "actual" values remains poorly understood and is likely to be material-specific. In many cases, the