Vapor Hydrogen Peroxide as Alternative to Dry Heat Microbial Reduction

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Abstract

The Jet Propulsion Laboratory, in conjunction with the NASA Planetary Protection Officer, has selected vapor phase hydrogen peroxide (VHP) sterilization process for continued development as a NASA approved sterilization technique for spacecraft subsystems and systems. The goal is to include this technique, with appropriate specification, in NPG8020.12C as a low temperature complementary technique to the dry heat sterilization process.

A series of experiments were conducted in vacuum to determine VHP process parameters that provided significant reductions in spore viability while allowing survival of sufficient spores for statistically significant enumeration. With this knowledge of D values, sensible margins can be applied in a planetary protection specification.

The outcome of this study provided an optimization of test sterilizer process conditions: VHP concentration, process duration, a process temperature range for which the worst case D value may be imposed, a process humidity range for which the worst case D value may be imposed, and robustness to selected spacecraft material substrates.

- **Objective:** introduce vapor hydrogen peroxide (VHP) as alternative to dry heat microbial reduction to meet planetary protection (PP) requirements

- **Goal:** to determine the minimum VHP vacuum process conditions for PP acceptable microbial reduction levels

  - Typical healthcare industries
    - The more kill, the better
    - High doses do not typically impact product
    - Sterility usually (destructively) tested by lot samples (many pieces in lot)

  - Flight Hardware
    - High doses may degrade performance
    - High doses may compromise material compatibility
    - One or two pieces in the "lot"

Overview

- **Four Experiment Sets:**
  1 - Concentration-time
  2 - Temperature
  3 - Humidity
  4 - Material Substrate

Steris MD2000 sterilizer

- **For Experiments 1, 2, 3: stainless steel coupons were deposited with 10^6 *G. stearothermophilus* spores (ATCC 7953), dried, and individually packaged in Tyvek/Mylar pouches.**

- **For Experiment 4: 10^6 spores (from the same spore suspension) were deposited directly on the five spacecraft materials, then individually packaged in Tyvek/Mylar pouches.**

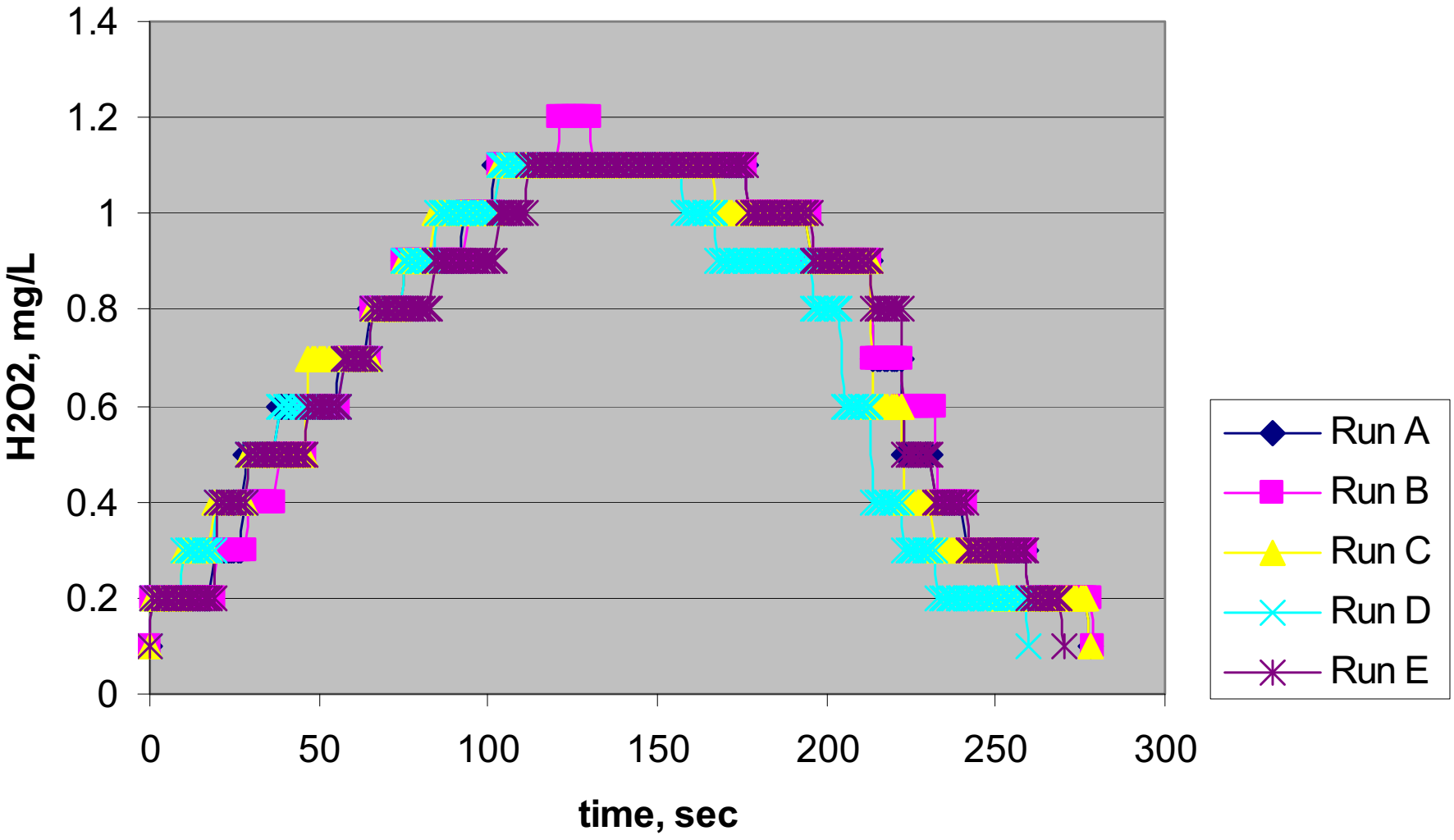
1 - Concentration-Time Effect on Lethality

- 2 nominal concentrations: 0.5 and 1.1 mg/L H2O2
- 2 nominal durations: one 1-minute; two 1-minute
- Fixed temperature: 35°C
- Fixed relative humidity: 5.5%
- Exposure pressure: 1 to 11 torr
- 5 replicate runs
- 5 stainless steel BIs per run
- Actual concentration vs time measured by a NIR H2O2 Vapor Monitor by Guided Wave Inc.

- **Results: Most survivors at 1.1 mg/L H_2O_2, one 1-minute duration**

Typical Concentration-Time Plot

as measured by Guided Wave NIR H2O2 Vapor Monitor

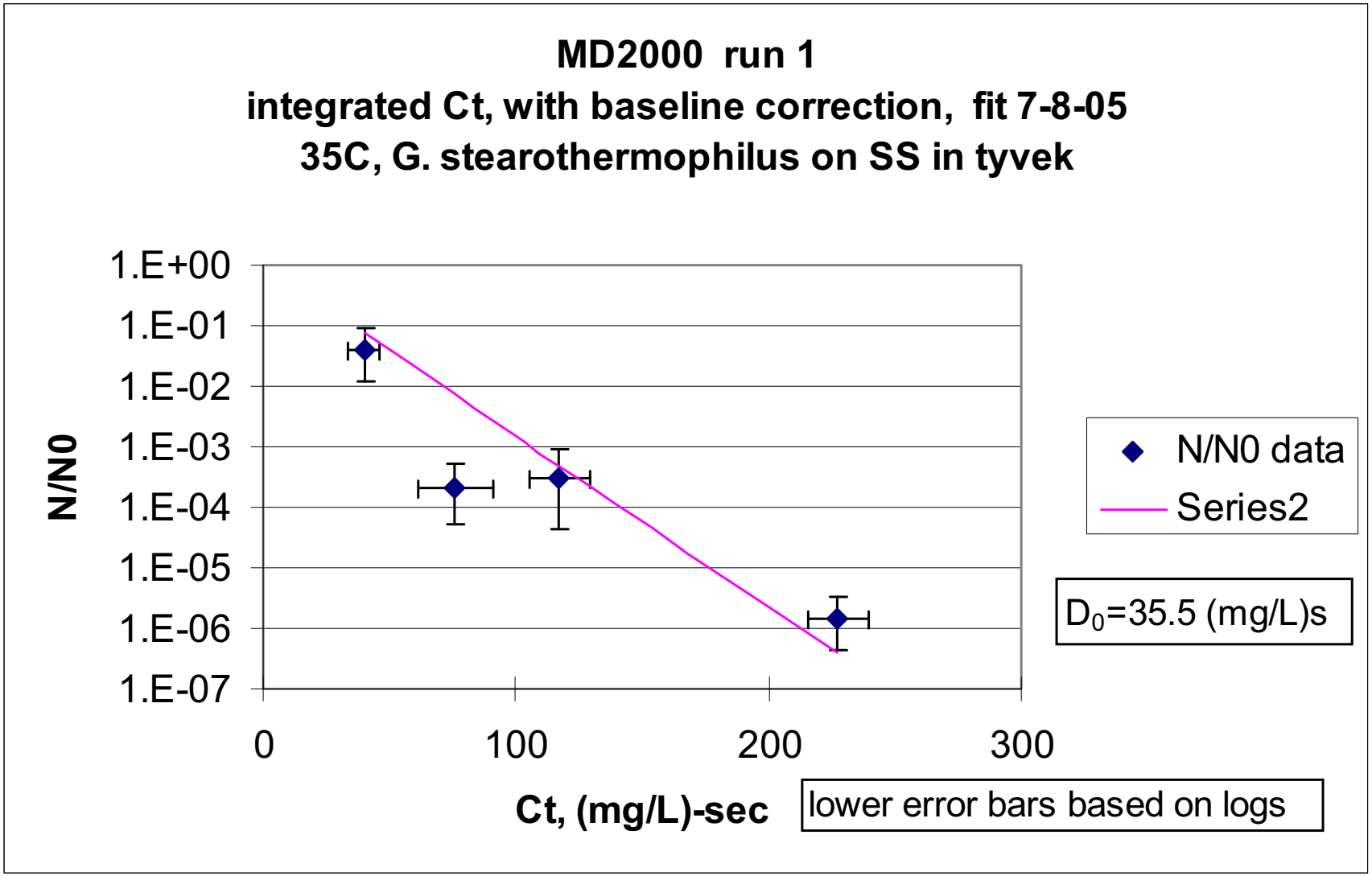


Simple Model

- To predict sub-lethal conditions to obtain data for real model construction
- Expect usual first order kinetics of lethality
- Expect lethality rate proportional to concentration C
- Expect lethality rate otherwise constant with time t (no explicit time dependence)
- N/No is the survival fraction
- Do is the D value (exposure Ct required to reduce the population by a factor of ten)

$$N/N_o = \exp(-Ct/D_o)$$

1 - Concentration-Time Effect on Lethality

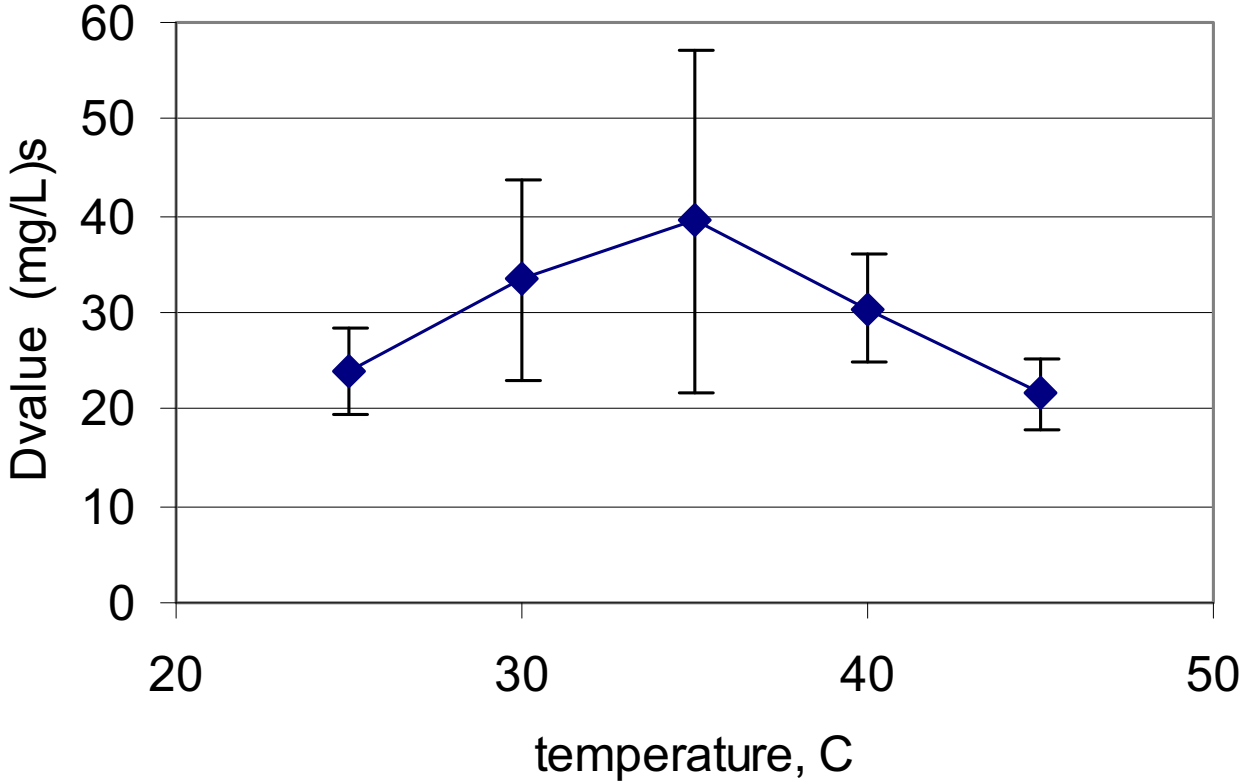


2 - Temperature Effect on Lethality

- Five temperatures: 25, 30, 35, 40, 45°C
- Fixed nominal concentration: 1.1 mg/L H2O2
- Fixed nominal duration: one 1-minute pulse
- Fixed relative humidity: 5.5%
- Exposure pressure: 1 to 11 torr
- 5 replicate runs
- 5 stainless steel BIs per run

- **Results: Most survivors at 35°C**
  (effect adjacent pair-wise significant at 0.9)

2 - Temperature Effect on Lethality

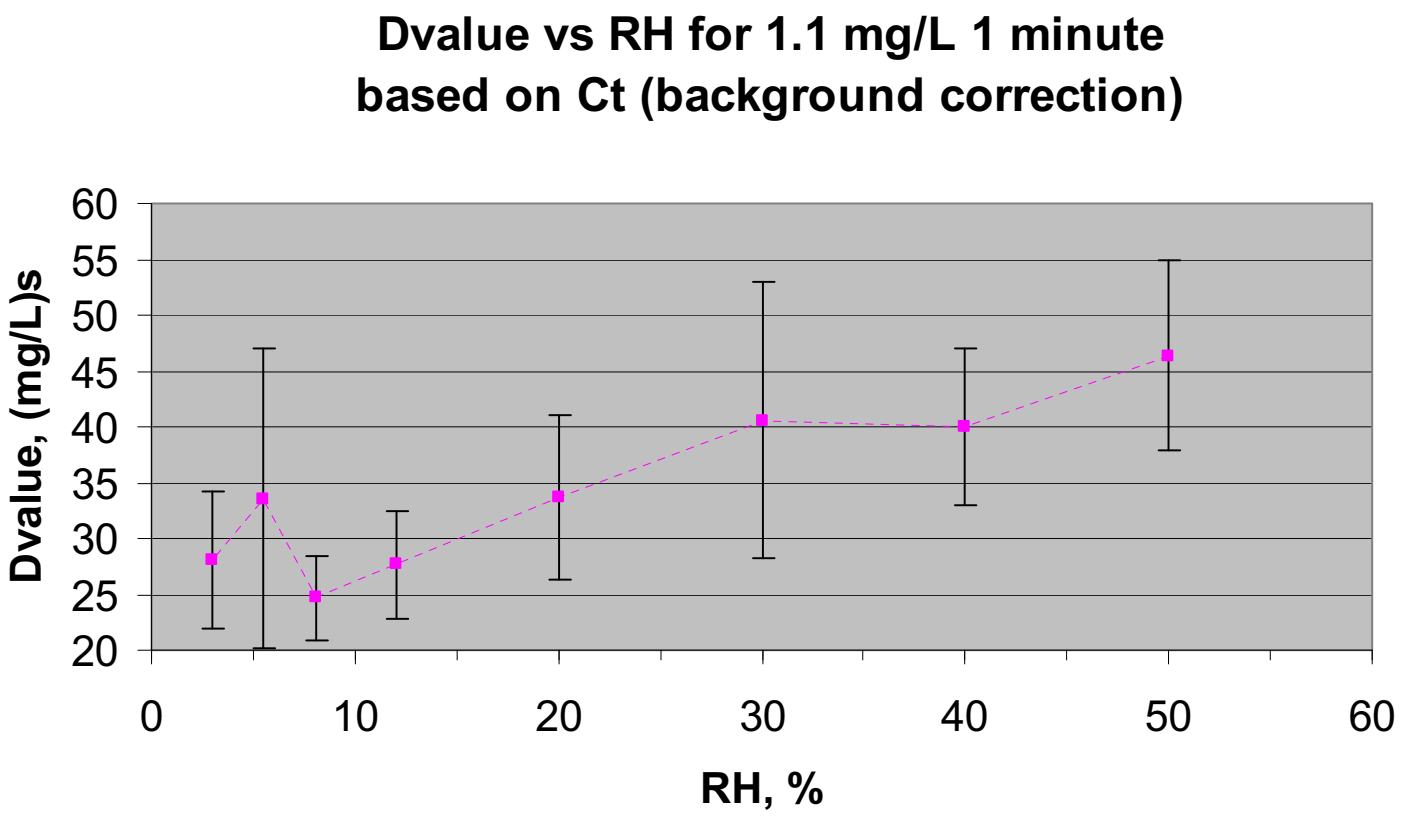


3 - Humidity Effect on Lethality

- Relative humidity: 3, 5.5, 8, 12, 20, 30, 40, 50%
- Fixed temperature: 35°C
- Fixed nominal concentration: 1.1 mg/L H_2O_2
- Fixed nominal duration: one 1-minute pulse
- Exposure pressure: 1 to 11 torr
- 5 replicate runs
- 5 stainless steel BIs per run

- **Results: Most survivors at 50%RH**
  (effect adjacent pair-wise significant at 0.9)

3 - Humidity Effect on Lethality



4 - Material Substrate Effect on Lethality

13x13mm

Bare Aluminum 6061

Graphite Composite

Kapton

Aptek 2711 White Paint

Z306 Black Paint

4 - Material Substrate Effect on Lethality

UV sterilization prior to spore deposition

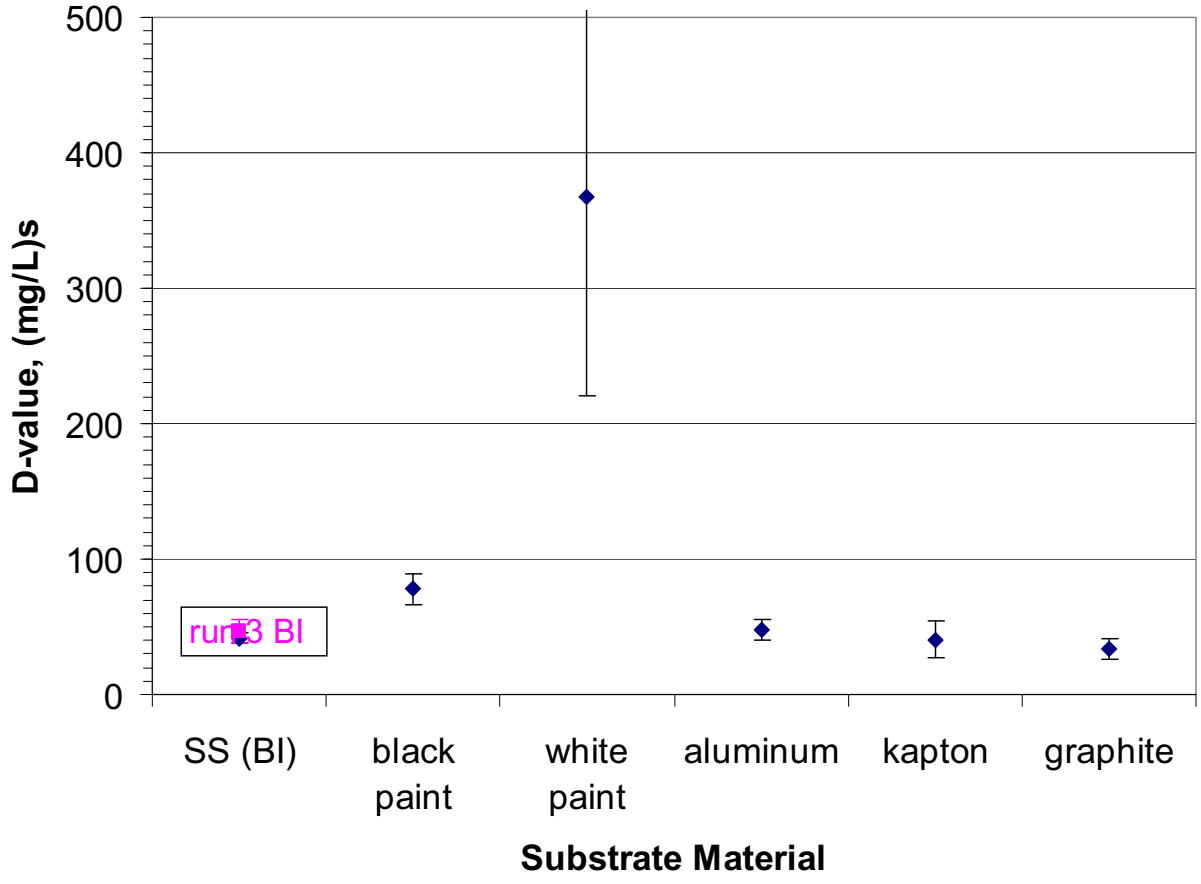
Ultra Violet Products CL-1000 Cross-Linker chamber

4 - Material Substrate Effect on Lethality

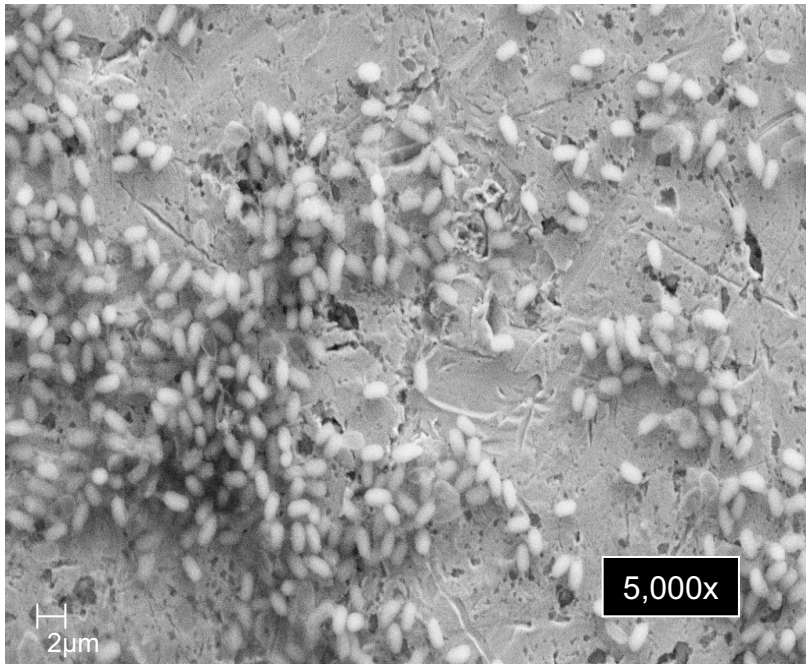
- Five common spacecraft materials: Z306, Aptek 2711, Kapton, aluminum, graphite composite
- Fixed relative humidity: 50%
- Fixed temperature: 35°C
- Fixed nominal concentration: 1.1 mg/L H_2O_2
- Fixed nominal duration: one 1-minute pulse
- Exposure pressure: 1 to 11 torr
- 5 replicate runs per material
- 5 coupons per run

- **Results: Most survivors : white paint > black paint > aluminum > Kapton > graphite composite**
  (only white paint significant at 0.9)

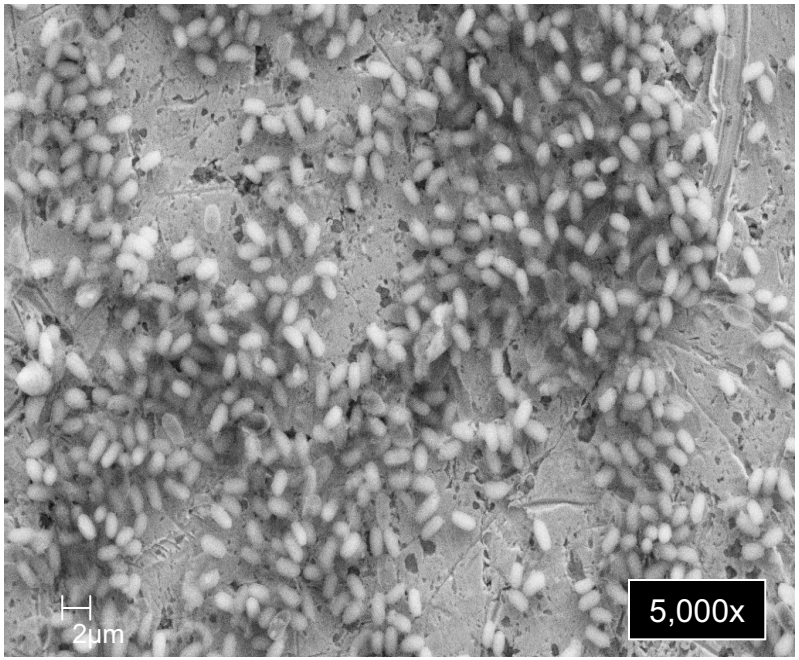
4 - Material Substrate Effect on Lethality



SEM photo of stainless steel coupons inoculated with 10E6 *G. stearothermophilus* spores

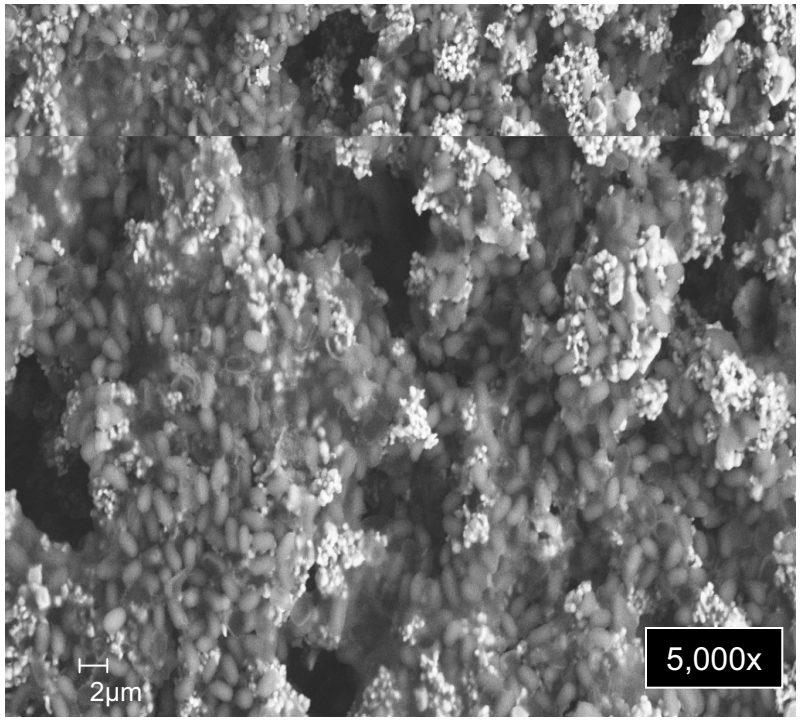


Un-exposed control

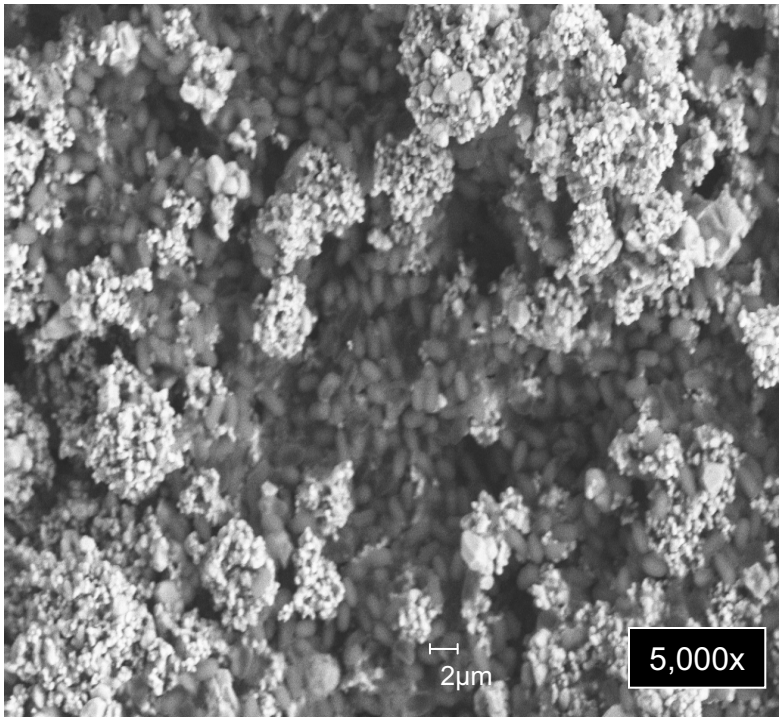


VHP-exposed

SEM photo of white paint coupons inoculated with 10E6 *G. stearothermophilus* spores



Un-exposed control



VHP-exposed

Summary

- Temperature effect (25-45°C): VHP least effective at 35°C (effect significant at 0.9)
- Humidity effect at 35°C (3-50%RH): least effective at 50%RH (significant at 0.9)
- Substrate effect at 35°C, 50%RH: least effective with White Paint, followed by Black Paint (only white paint significant at 0.9)

- *Possible* PP Specification based on these results:
  - In vacuum, the D value for hydrogen peroxide reduction of surface spore burden is 100 (mg/L)s *(plus any additional margin desired). This value bounds the results for the environmental parameters specified below and for all substrates, except the white paint.*
  - The exposure shall be measured in real time for the vapor concentration of hydrogen peroxide, no lower than 0.5 mg/L.
  - The temperature shall be in the range 25 to 45C. The absolute humidity shall be in the range 0.22 g/L to 1.1 g/L (corresponding to an RH of 10 to 50% at 35C).

Conclusions

- D-value is a better statistic than survival fraction.
- Bounding conditions for the D-value for useful ranges of temperature and humidity were obtained.
- The use of sub-lethal conditions permitted modeling, statistical comparisons, and minimum conditions for kill. (Reasonable margins may be applied later.)
- Direct measurement of H2O2 concentration vs. duration is essential.
- Only one substrate material (white paint) showed any statistically significant effect. It may be real or an artifact of depositing an aqueous suspension (i.e, surface energy effect), which is *not* realistic for spacecraft surfaces.