Experimental Modeling Of Sterilization Effects Of Atmospheric Entry Heating On Bacterial spores of *Bacillus atrophaeus*

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Planetary Protection Technology Survey for the Proposed Mars Sample Return Mission

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Date 8/10/11

Bacillus atrophaeus

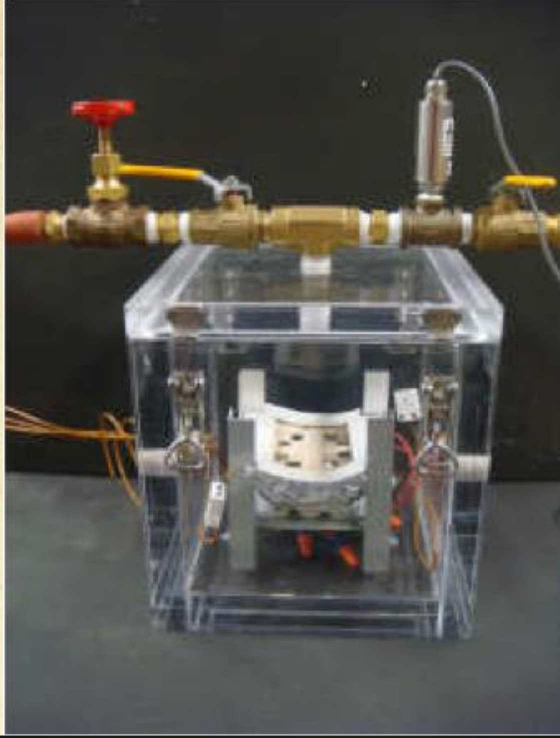
- Gram-positive bacteria: thick peptidoglycan
- Found in soil
- Spore forming bacteria
- Tolerate extreme environment conditions
- Indicator for the evaluation of dry heat sterilization procedures

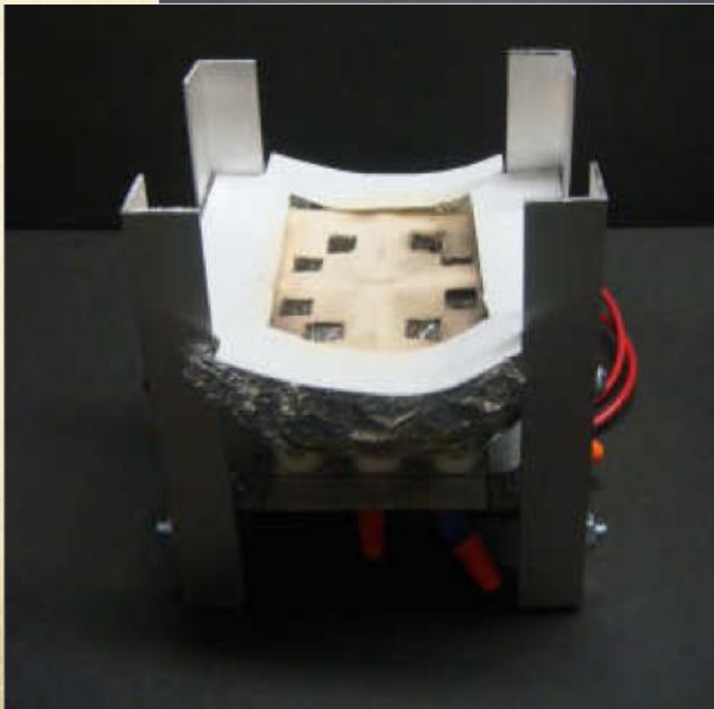
Research Question

- To find the critical temperature of spore inactivation
- To find the critical rate of heat inactivation of *Bacillus atrophaeus spores* that attached to spacecraft surfaces
- To evaluate the designed apparatus

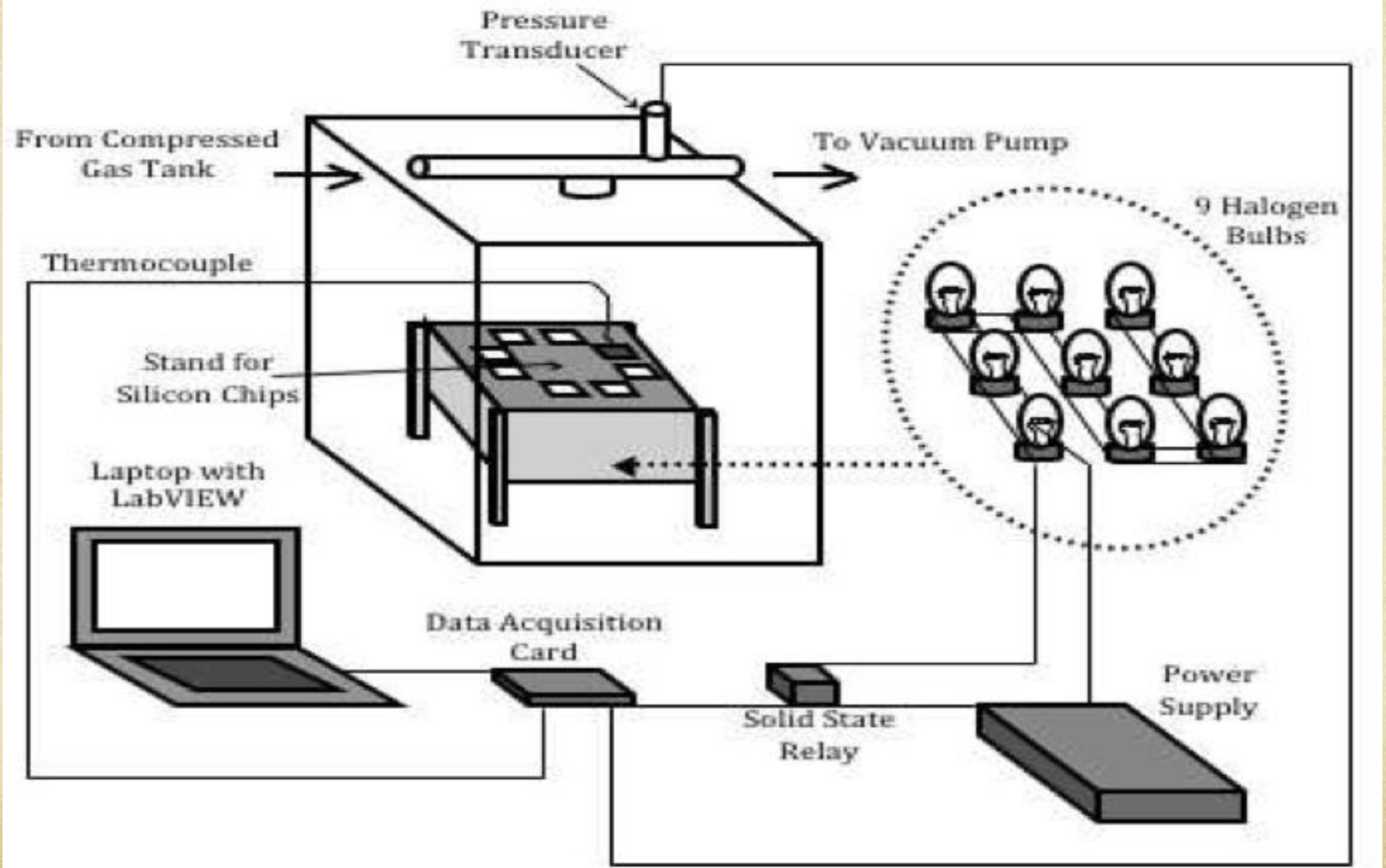
Materials

- To test atmospheric heating profiles
- Provide a method to control the heat exposure and temperature profile that bacteria spores would experience
- Control complex-shaped heating profiles for temperatures from ambient to nearly 500° C
- Vacuum or special atmospheric gas mixes
- Heating by tungsten-halogen lamps
- Controlled by LabView
- Temperature of the substrate monitored in each run





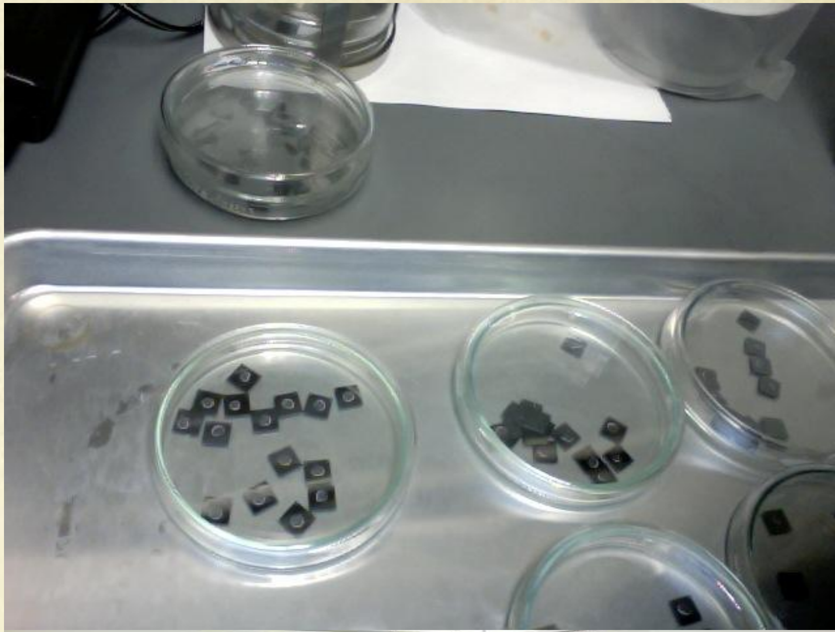
Design

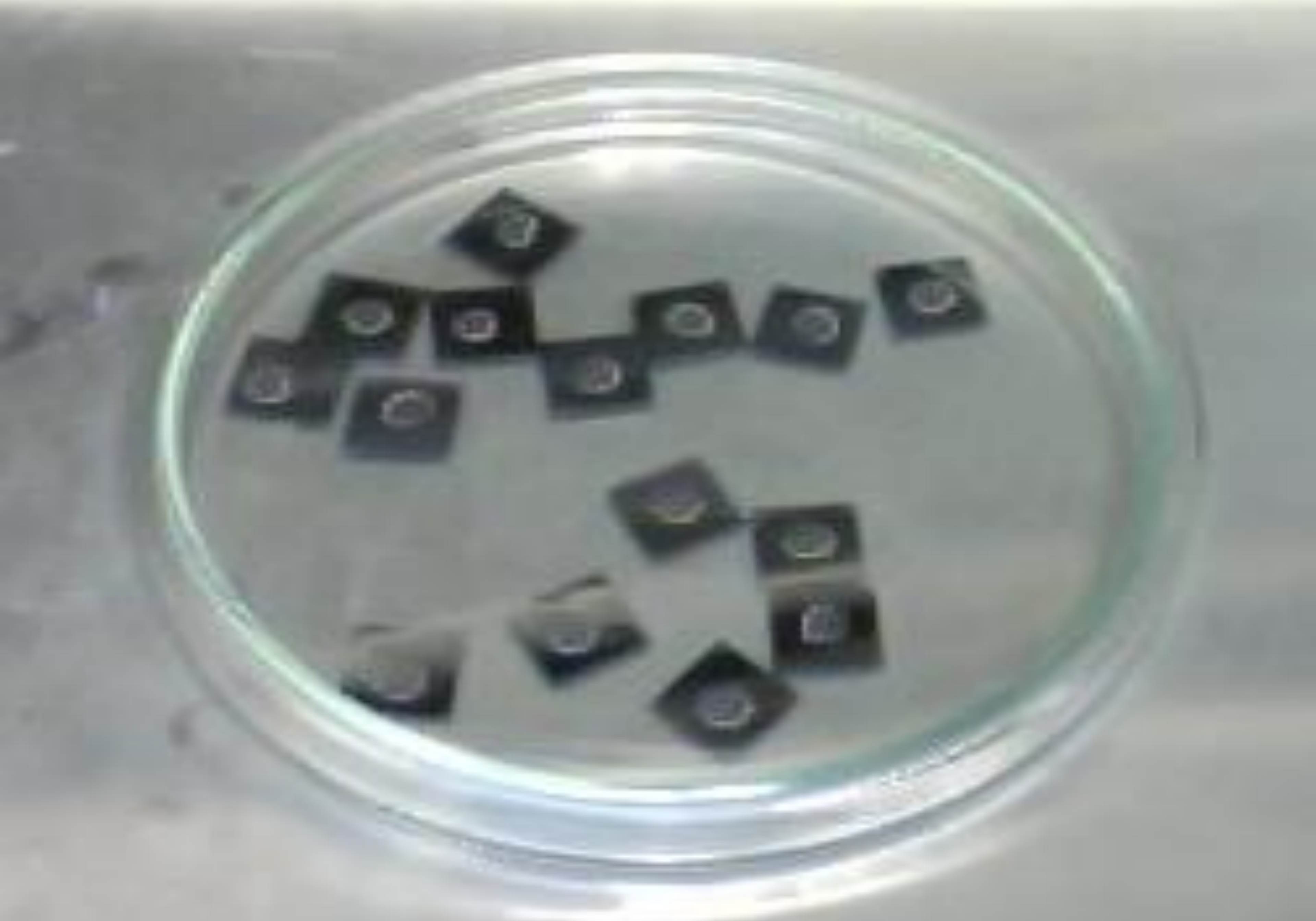


Pressure Transducer
From Compressed Gas Tank
To Vacuum Pump
9 Halogen Bulbs
Thermocouple
Stand for Silicon Chips
Laptop with LabVIEW
Data Acquisition Card
Power Supply
Solid State Relay

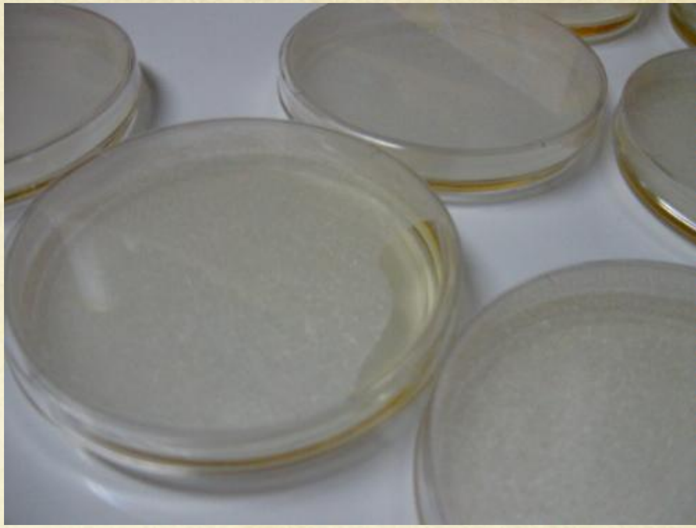
Preparing Silicon Chips

- Cleaning with Isopropanol and Acetate
- Pre-sterilize chips:
  - 170 °C for one hour in oven
- Pipetting 10 µL of diluted *Bacillus atrophaeus* spores $(9*10^5)$
- Incubate overnight
- Dried out over saturated Lithium chloride [controls relative humidity at 15%]



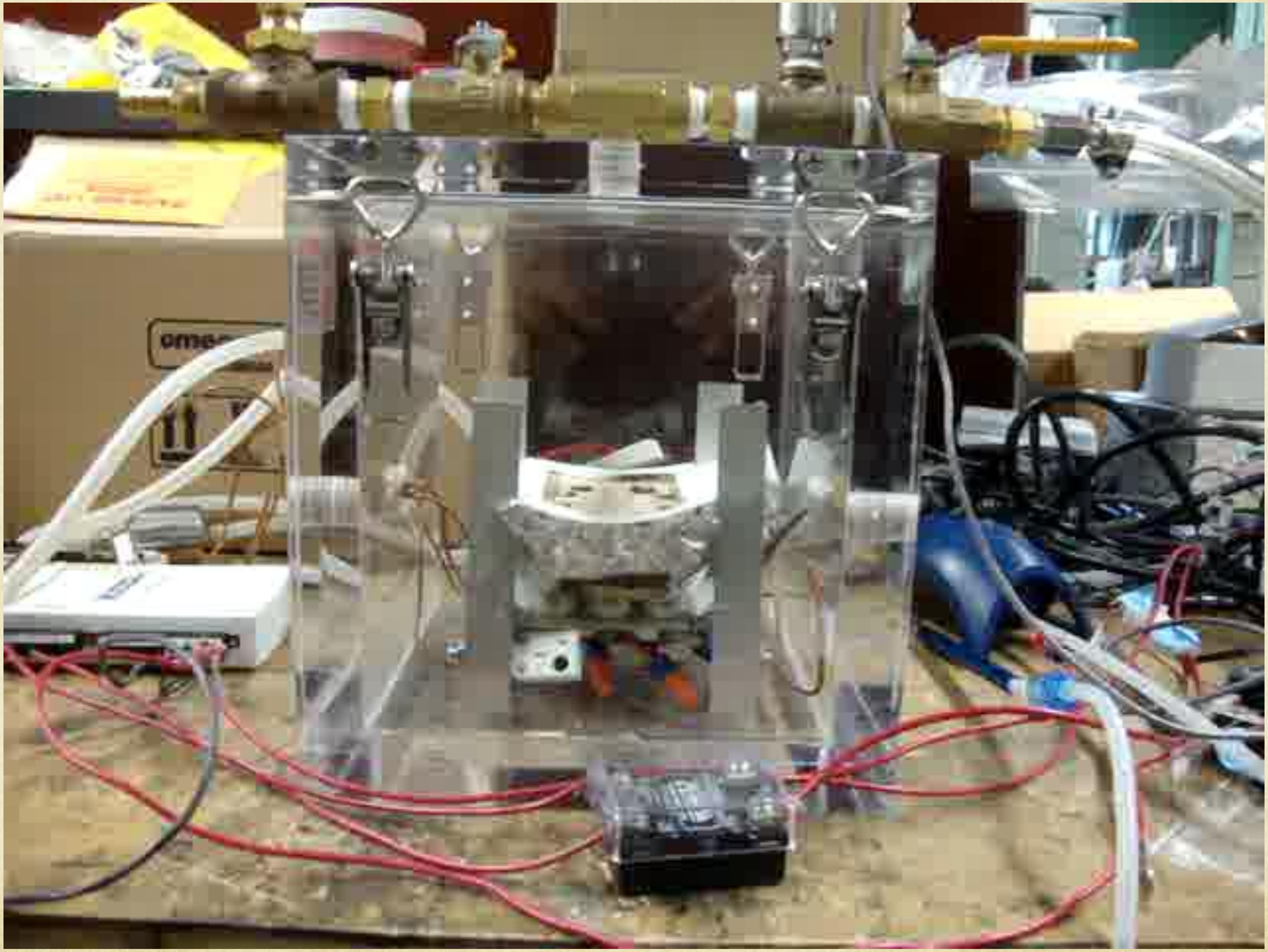


Dilution and plate counting



- 6 fold dilution
- Plating
- Counting the colonies

Actual Run



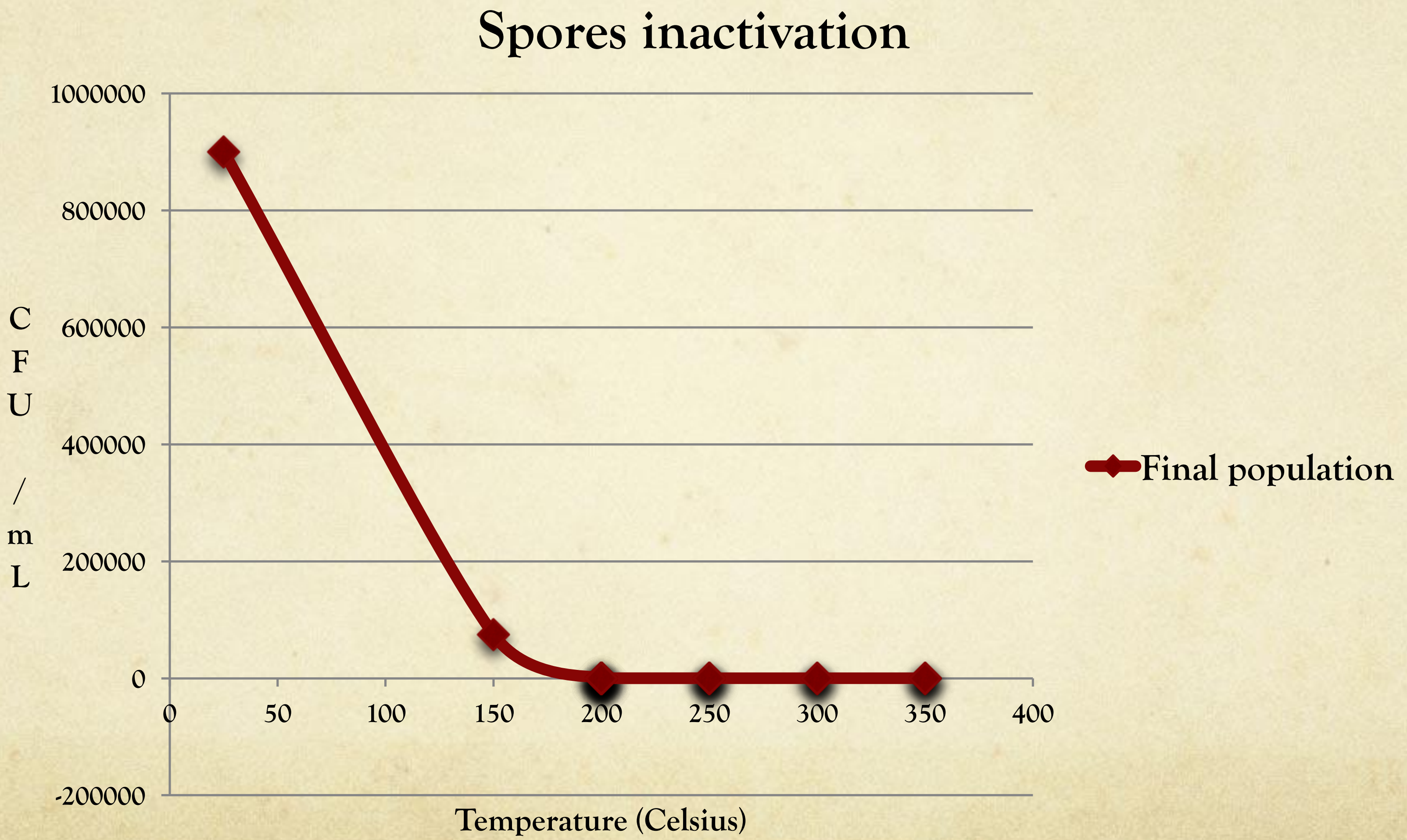
Results

Run #	Initial population	Time	Temp.	Atmosphere	Final population
1	1.6 10^8	180 s	150 ° C	Ambient	75000/ml
2	1.6 10^8	360 s	150 ° C	Ambient	0
3	1.6 10^8	30 s	200 ° C	Ambient	0

Results

Run #	Initial population	Time	Temperature	Atmosphere	Final population
4	1.6 10^{8}	180 s	200 ° C	Ambient	0
5	1.6 10^{8}	360 s	200 ° C	Ambient	0
6	1.6 10^{8}	30 s	250 ° C	Ambient	0
7	1.6 10^{8}	180 s	250 ° C	Ambient	0
8	1.6 10^{8}	360 s	250 ° C	Ambient	0
9	1.6 10^{8}	30 s	300 ° C	Ambient	0
10	1.6 10^{8}	180 s	300 ° C	Ambient	0
11	1.6 10^{8}	360 s	300 ° C	Ambient	0
12	1.6 10^{8}	30 s	350° C	Ambient	0
13	1.6 10^{8}	180 s	350° C	Ambient	0
14	1.6 10^{8}	360 s	350 ° C	Ambient	0
15	1.6 10^{8}	180 s	150 ° C	vacuum	0
16	1.6 10^{8}	360 s	150 ° C	vacuum	0
17	1.6 10^{8}	30 s	200 ° C	Ambient	0
18	1.6 10^{8}	30 s	200 ° C	vacuum	0
19	1.6 10^{8}	180 s	200 ° C	vacuum	0
20	1.6 10^{8}	360 s	200 ° C	vacuum	0
21	1.6 10^{8}	30 s	250 ° C	vacuum	0
22	1.6 10^{8}	180 s	250° C	vacuum	0
23	1.6 10^{8}	30 s	300 ° C	vacuum	0
24	1.6 10^{8}	180 s	300 ° C	vacuum	0
25	1.6 10^{8}	30 s	350 ° C	vacuum	0
26	1.6 10^{8}	180 s	350 ° C	vacuum	0

Results



Spores inactivation
1000000
800000
600000
400000
200000
0
-200000
C F U / m L
0
50
100
150
200
250
300
350
400
Temperature (Celsius)
Final population

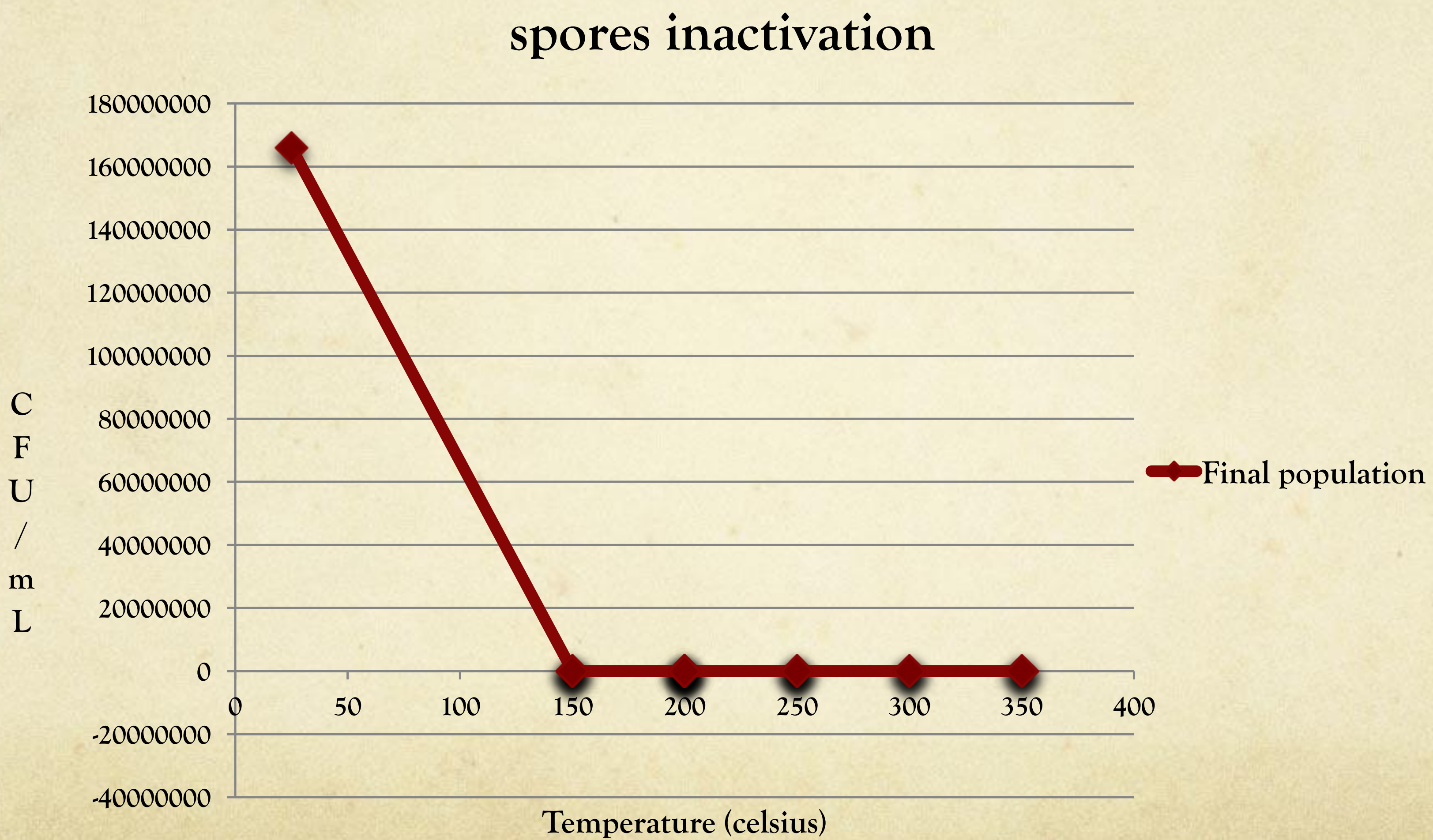
Experiment summary

- Spore survived at 150 °C in 180 seconds
- No growth at 150 °C in 360 seconds and above
- Most spores are inactivated/killed under these conditions

Results

Run #	Initial population	Time(S)	Temperature	Atmosphere	Final population
1	1.6 10^{8}	15	150 ° C	Ambient	0
2	1.6 10^{8}	60	150 ° C	Ambient	0
3	1.6 10^{8}	120	200 ° C	Ambient	0
4	1.6 10^{8}	180	200 ° C	Ambient	0
5	1.6 10^{8}	15	200 ° C	vacuum	0
6	1.6 10^{8}	60	250 ° C	Vacuum	0
7	1.6 10^{8}	120	250 ° C	Vacuum	0
8	1.6 10^{8}	180	250 ° C	Vacuum	0
9	1.6 10^{8}	15	300 ° C	Ambient	0
10	1.6 10^{8}	15	300 ° C	Ambient	0
11	1.6 10^{8}	15	300 ° C	Ambient	0
12	1.6 10^{8}	15	350° C	Ambient	0
13	1.6 10^{8}	15	350° C	Ambient	0
14	1.6 10^{8}	15	350 ° C	Ambient	0
15	1.6 10^{8}	20	150 ° C	Ambient	0
16	1.6 10^{8}	25	150 ° C	Ambient	0
17	1.6 10^{8}	30	200 ° C	Ambient	0
18	1.6 10^{8}	10	200 ° C	Ambient	0
19	1.6 10^{8}	20	200 ° C	Ambient	0
20	1.6 10^{8}	25	200 ° C	Ambient	0
21	1.6 10^{8}	15	250 ° C	Ambient	0

Results

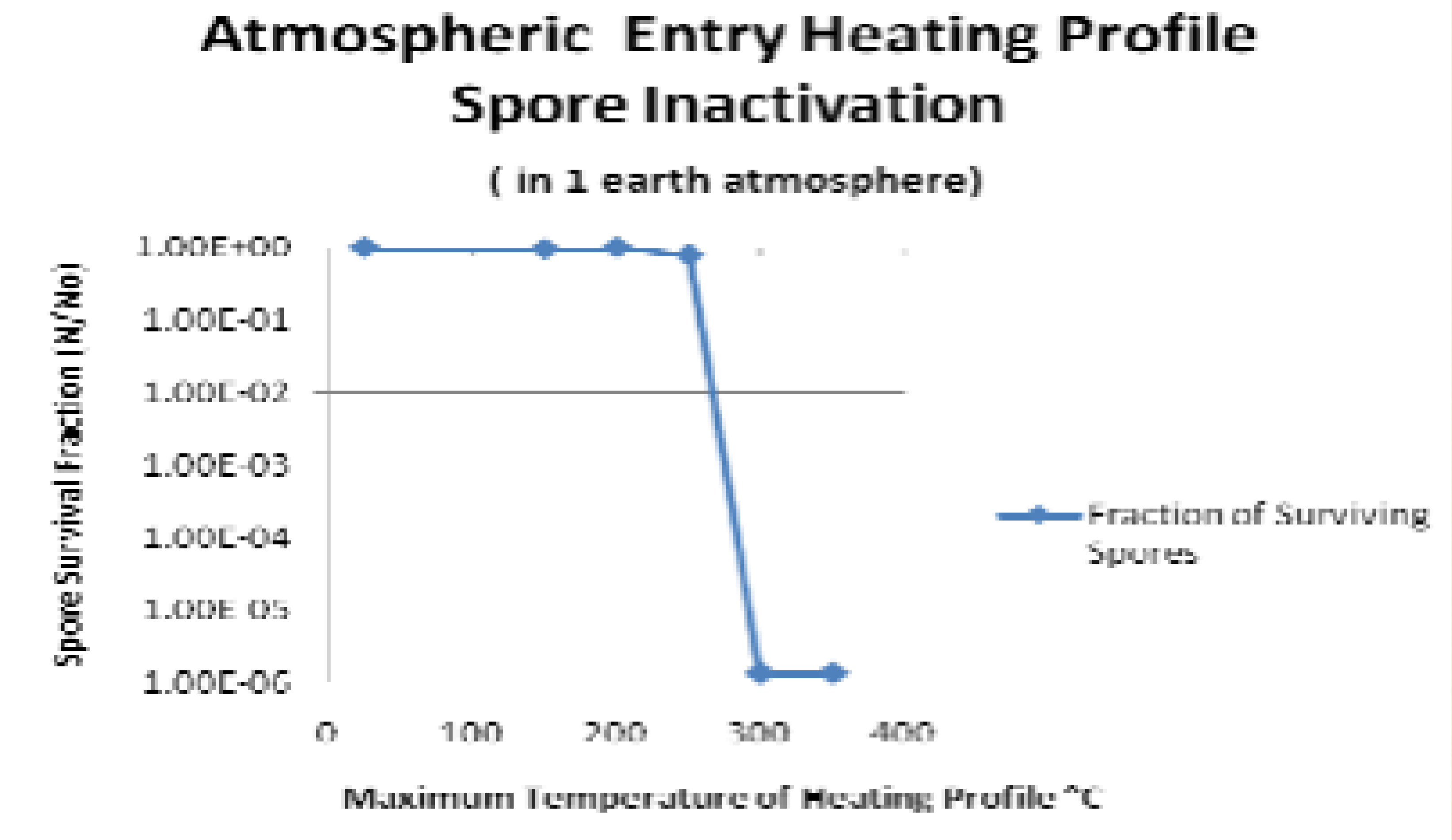


spores inactivation
180000000
160000000
140000000
120000000
100000000
80000000
60000000
40000000
20000000
0
-20000000
-40000000
0
50
100
150
200
250
300
350
400
C F U / m L
Temperature (celsius)
Final population

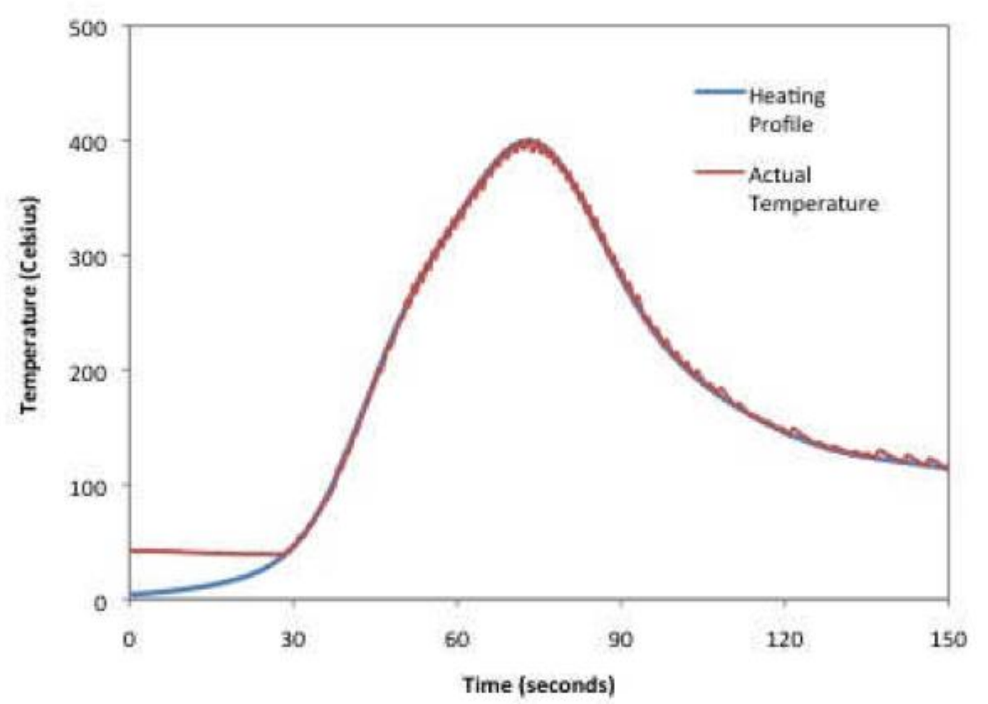
Experiment summary

- Nearly all spores were inactivated
- Spores of *Bacillus atrophaeus* inactivate at 150 °C in 15 seconds and above
- Inactivation of spores occurred in shorter period of time (15 seconds) compare to the previous experiment (180 s)
  - Fast heat-up rate influenced the kill rate of the spores

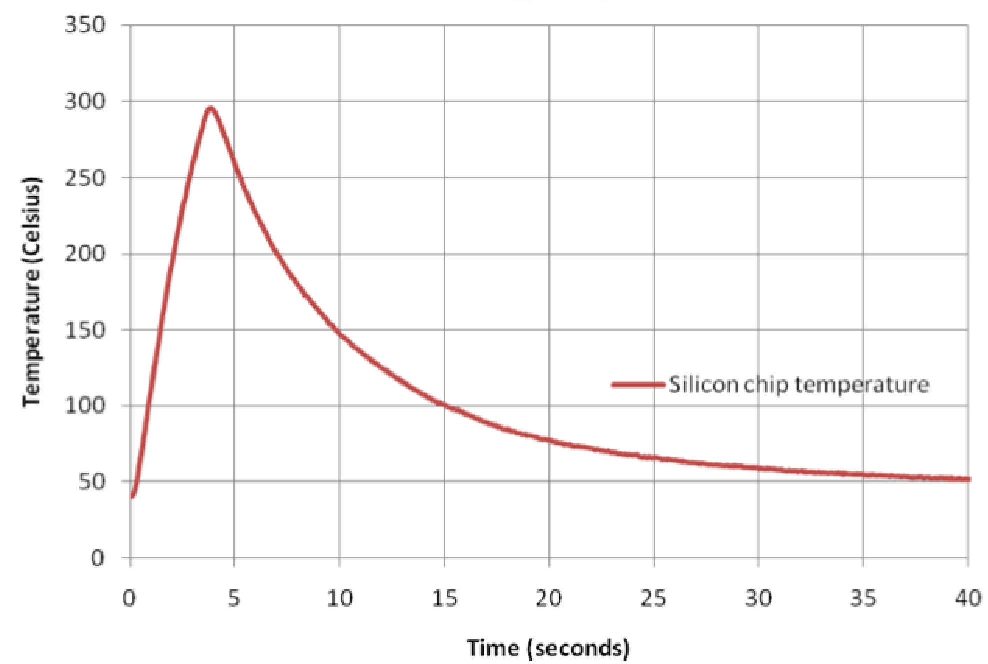
Heating profile plot



Atmospheric heating profile

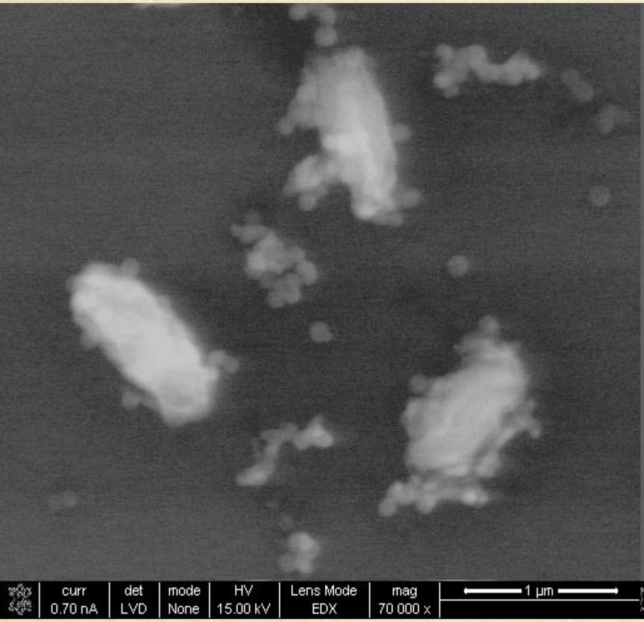


Rapid heating spike profile

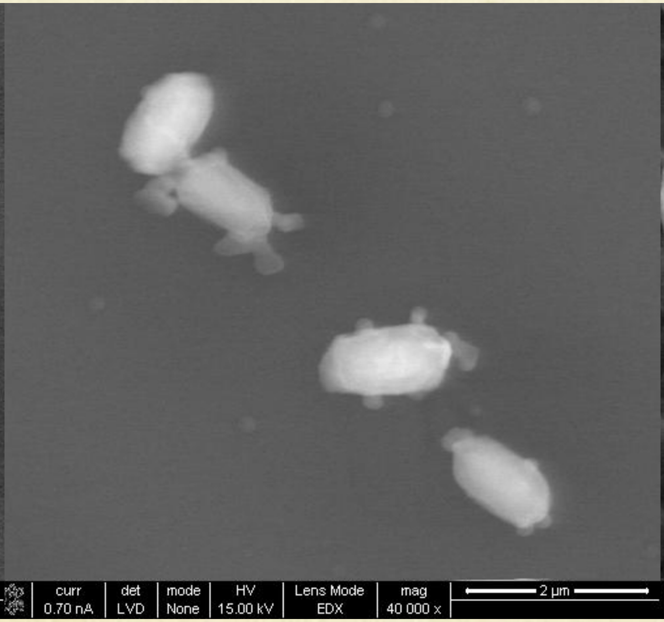


scanning electron microscope images of Bacillus atrophaeus

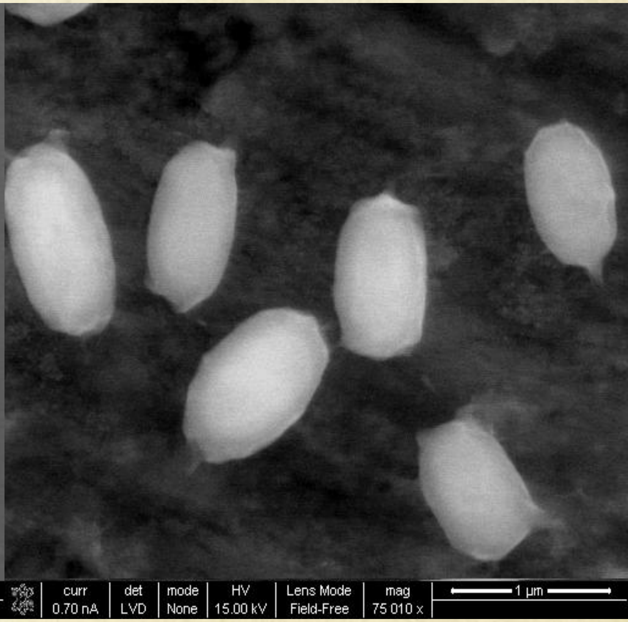
Temperature of 450 C



Temperature of 350 C



Normal



Conclusion

- The designed apparatus was able to evaluate *Bacillus atrophaeus* and spore inactivation
- Bacillus *atrophaeus* spores inactivate at 150 °C in 15 seconds (rapid heating)
- Bacillus *atrophaeus* spores inactivate between 250 and 300 (slow heating)
- Fast heat-up rate increases the killing rate of the spores

Part II of summer work

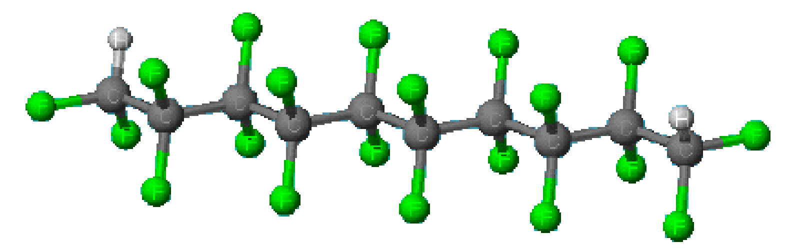
Planetary Protection Technology Survey for the Proposed Mars Sample Return Mission

Survey for the Proposed Mars Sample Return Mission

- Evaluating potential material for sample container hardware
- Maintaining material integrity on Mars for long period of time
- Not introducing terrestrial contaminant to the samples
- evaluating the advantage and disadvantage of using Teflon

Teflon

- Non-stick
- Heat resistance
- Cryogenic stability
- Chemical resistance, reactive with Alkali metals, inter-halogen compounds, sodium potassium alloy
- Non-wetting
- Low coefficient of friction
- Unique electrical properties
- Excellent optical properties
- Weather and UV resistance



Teflon, $-(CF_2CF_2)-$

Types of Teflon

- Teflon PTFE
- Teflon PFA
- **Teflon FEP (fluorinated ethylene propylene)**
- Teflon NXT
- Teflon AF
- Zonyl PTFE
- Tefzel ETFE Resin
- Teflon Dry Lubricant
- Teflon One Coat

Properties of Teflon

Properties	Teflon PTFE	Teflon (FEP)	Teflon PFA	Tefzel
Specific gravity	2.13-2.22	2.15	2.15	1.70-1.78
Tensile Strength Mpa	21-35 Mpa	23 Mpa	25 Mpa	40-47 Mpa
Elongation %	300-500%	325%	300%	150-300%
Flexural Modulus Mpa	500 Mpa	600 Mpa	600 Mpa	1,000 Mpa
Folding Endurance (MIT)	>106(MIT)	5-80 x 103	10-500 x 103	10-27 x 103
Impact Strength J/m	189 J/m	No Break	No Break	No Break
Hardness Shore D	50-65 Shore D	56 Shore D	60 Shore D	63-72 Shore D
Coefficient of Friction, Dynamic <3 m/min	0.1	0	0.2	0.23

Properties of Teflon

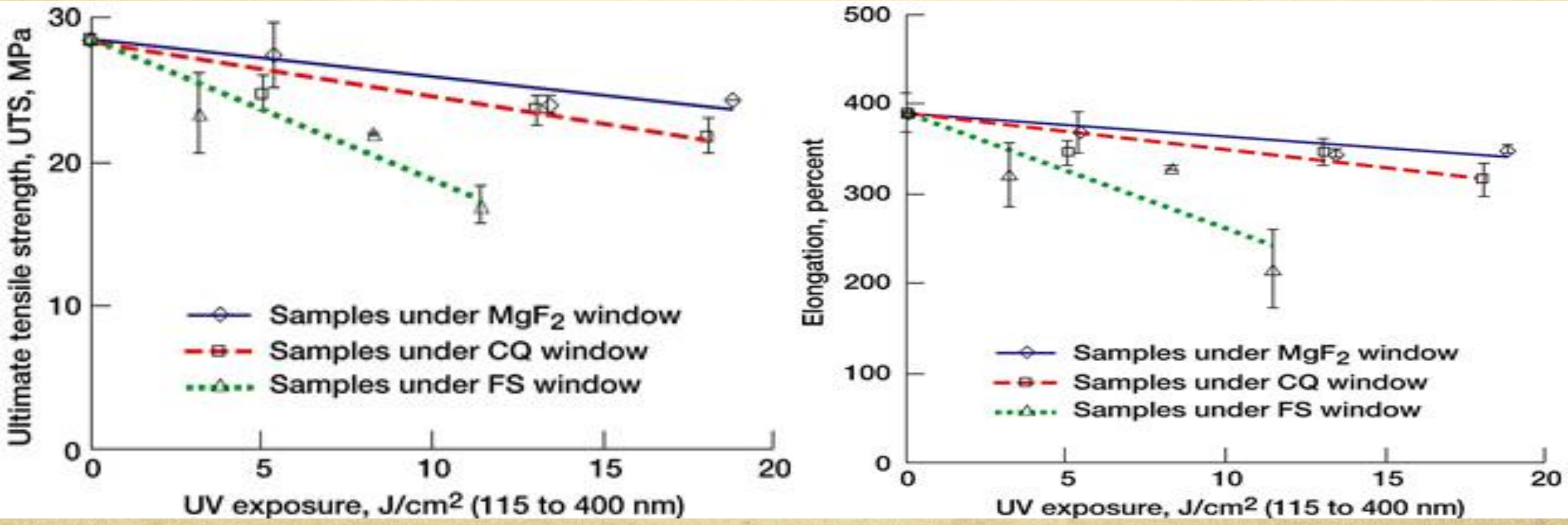
Properties	Teflon PTFE	Teflon (FEP)	Teflon PFA	Tefzel
Melting point °C (°F)	327 (621)	260 (500)	305 (582)	245-280 (473-536)
Upper Service Temperature (20,000h) °C (°F)	260(500)	204(400)	260(500)	155(311)
Limiting Oxygen Index %	>95	>95	>95	30-36
Heat of Combustion MJ/kg	5.1	5.1	5.3	13.7

Thermal decomposition products of Teflon

- Carbonyl fluoride
- Difluorophosgene
- Hexafluoroethane (Feron 116) (300-360 °C)
- Hydrogen fluoride
- Octafluorocyclobutane (300-360 °C)
- Perfluorisobutylene
- Fluorine monoxide (OF2)
- Perfluoroisobutylene (PFIB) (380-400 °C)
- Tetrafluoroethylene (TFE) (500-550 °C)

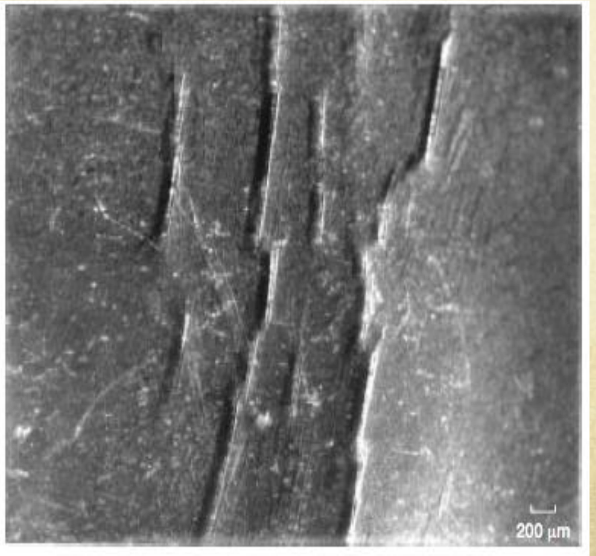
Teflon degradation

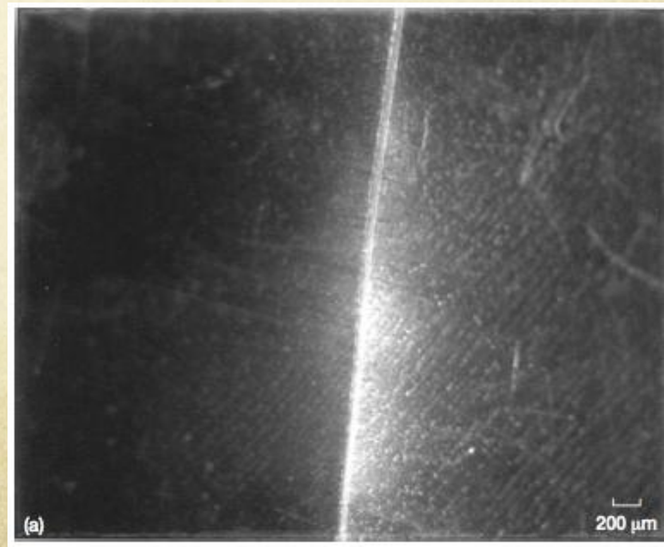
- Surface and Bulk Degradation of Teflon® FEP Retrieved from the Hubble Space Telescope Solar Arrays
  - Observed cracks in the FEP layers
    - Result of radiation and thermal effects



Conclusion

- Advantage: good physical and mechanical properties
- Disadvantages: Decomposition and degradation due to the radiation and thermal effects





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Extra slides

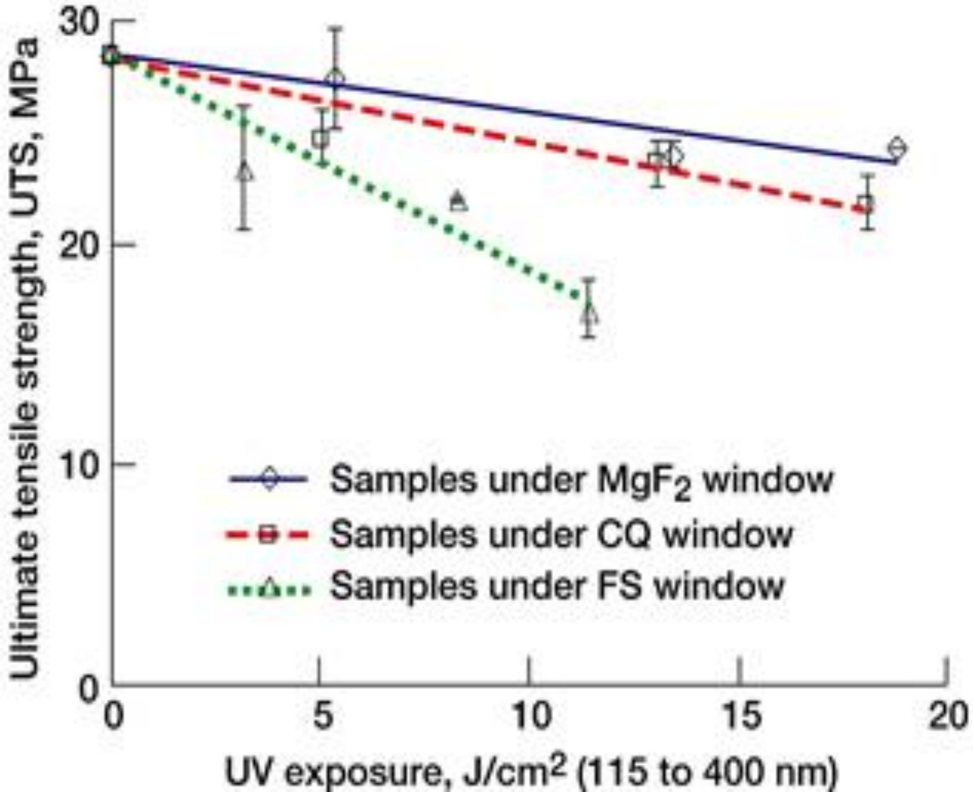
Surface and Bulk Degradation of Teflon® FEP Retrieved from the Hubble Space Telescope Solar Arrays

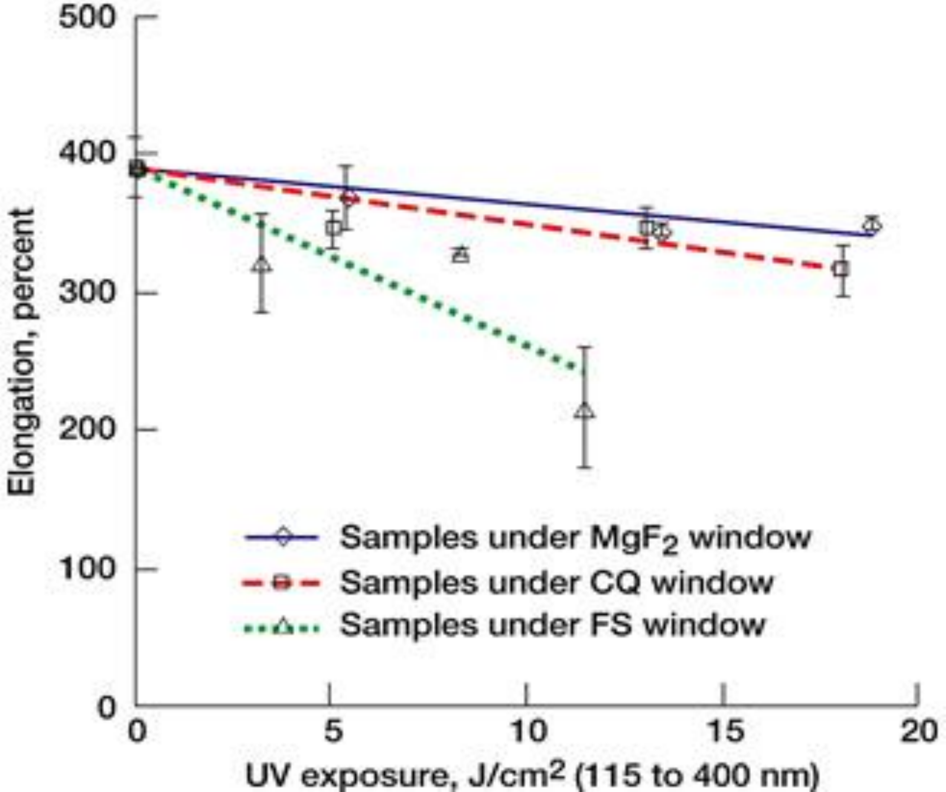
- Observed cracks in the FEP layers
  - Result of radiation and thermal effects
- Examine the effects of different wavelength ranges of vacuum ultraviolet (VUV) radiation on the degradation of the mechanical properties of FEP
- Teflon FEP film (50.8 Mm) exposed to radiation from a VUV lamp from beneath different cover windows
- MgF2 (115 to 400 nm), crystalline quartz (140 to 400), and fused silica (FS, 155 to 400 nm)

Ultraviolet exposure on the mechanical properties of Teflon FEP

Fastest rate of mechanical properties degradation is exposed to 155- to 400-nm wavelengths from beneath fused silica

Slower rate of degradation from beneath the CQ and MgF2 windows is due to fluence that only thins the material and does not lead to embrittlement and may remove an embrittled layer





Results

- VUV radiation of wavelength in 160 nm are absorbed significantly within a thin slice of the Teflon
- Resulting in a concentration of photoreactions near the surface
- Causing erosion
- Surface erosion occurred in broad spectrum VUV (115 to 400 nm) and monochromatic VUV (147 nm)
- Mechanical degradation was caused by 115-to 400 nm VUV
- It is likely that laboratory VUV exposure with these sources would cause greater surface erosion than would occur in space
  - Laboratory VUV peak is at 160 nm, which matches the absorption peak of Teflon but is not representative of a peak in the solar spectrum.

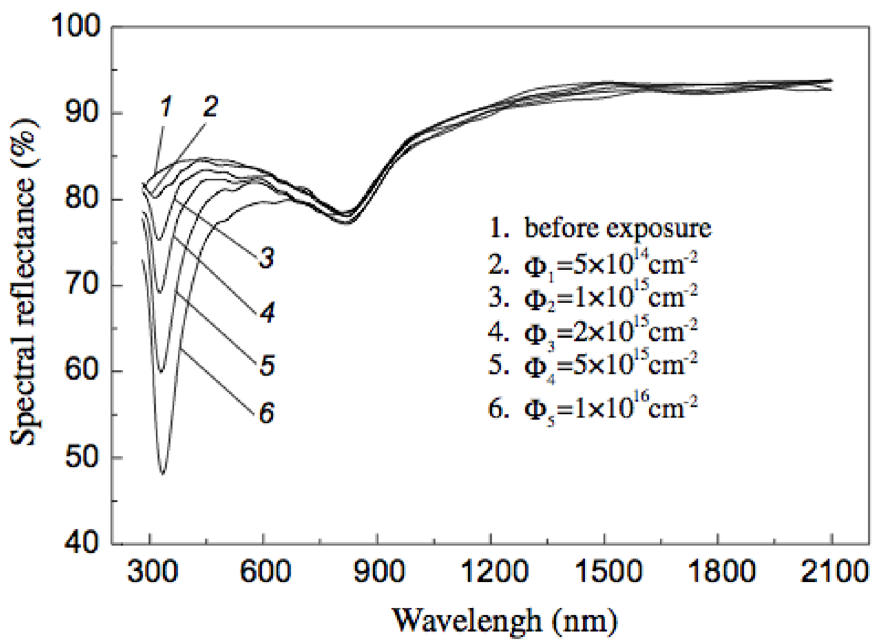
Effects of proton exposure on aluminized Teflon FEP film degradation

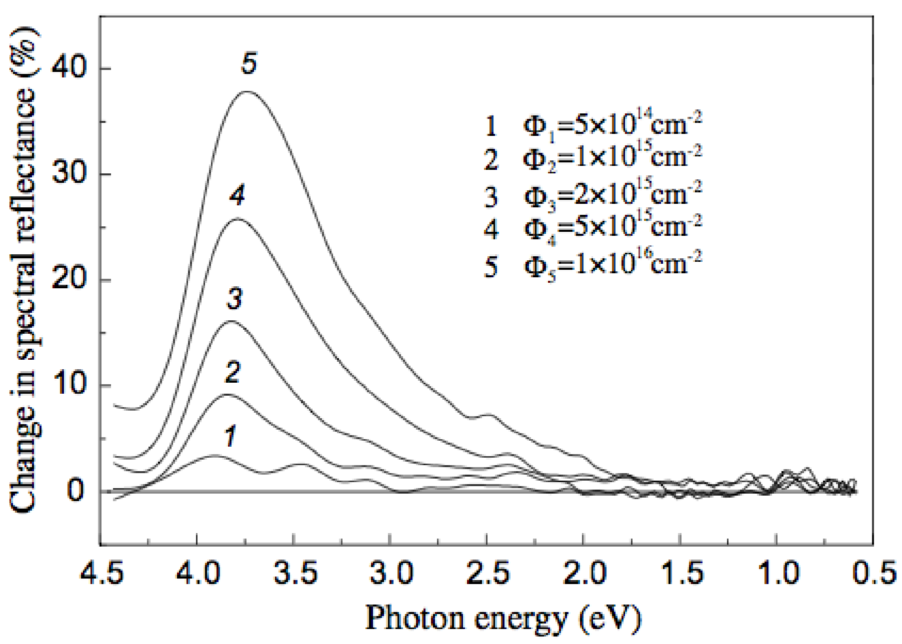
- Detecting degradation of Teflon FEP films under proton radiation
- Measuring the spectral reflectance of Teflon FEP/Al film before and after proton radiation
- Examining the microstructure of the specimens

Effects of proton exposure on aluminized Teflon FEP film degradation

The radiation with protons results in formation of an absorption band in the wavelength region of 280-600 nm

With increasing proton fluence, the absorption band was widened

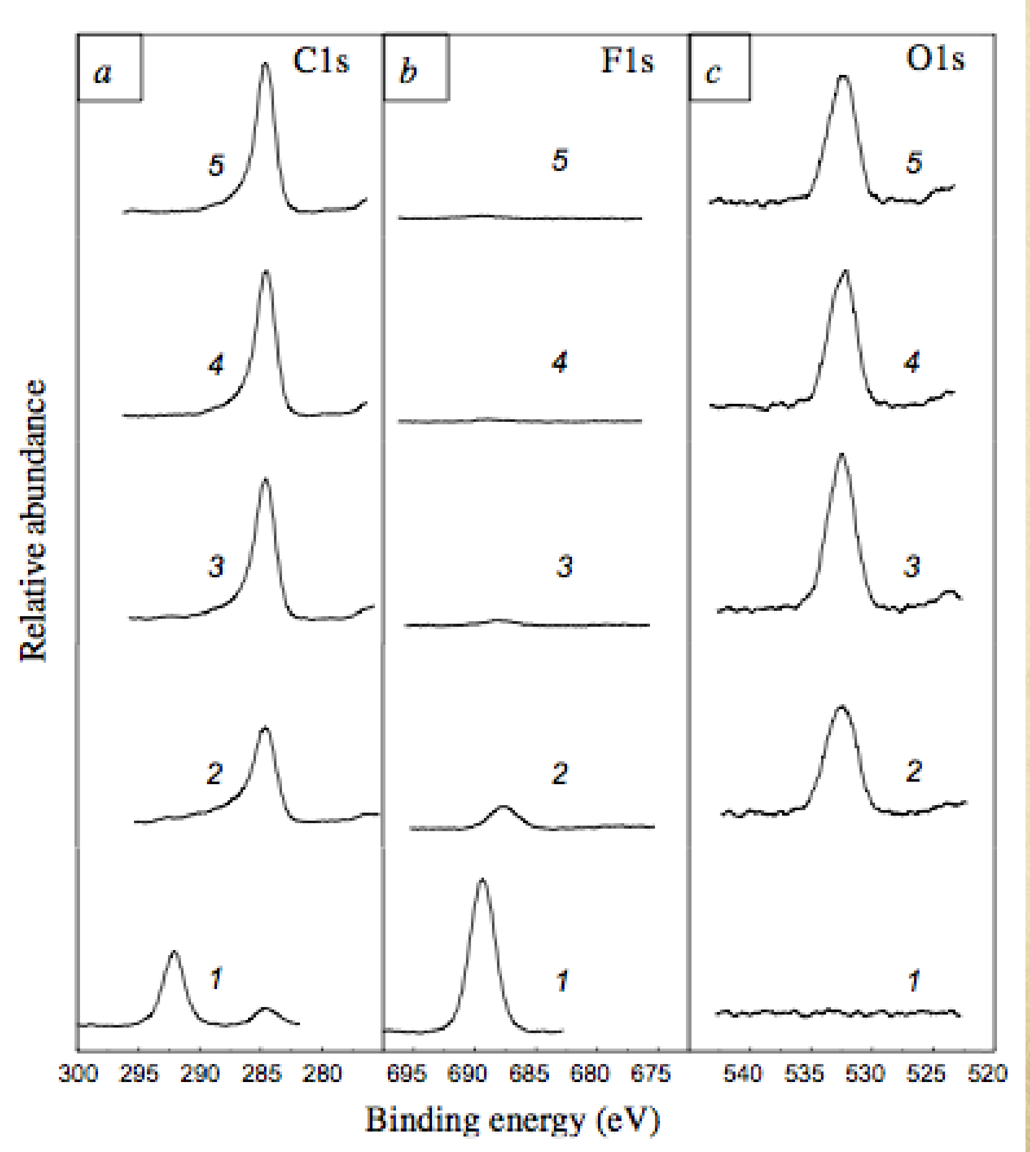




Effects of proton exposure on aluminized Teflon FEP film degradation

(1) Before radiation
(2) Radiated for 1×10 ^{15} cm $^{-2}$
(3) Radiated for 2 ×10 ^{15} cm $^{-2}$
(4) Radiated for 5 ×10 ^{15} cm $^{-2}$
(5) Radiated for 1 ×10 ^{15} cm $^{-2}$

Under proton exposure, the large molecules in Teflon FEP film were activated, and fluorine atoms and –CF3 groups could be bombarded out of the main chains to form free radicals and free carbon atoms in the surface layer of specimens



Mechanical Properties Degradation of Teflon® FEP Returned From the Hubble Space Telescope

- Bend testing
- Tensile testing
- Surface hardness measurement of Hubble Space Telescope MLI (multi-layer insulation) materials retrieved during SM1 (first servicing mission) and SM2 (second servicing mission)

HST environment

- Solar exposure
  - Near ultraviolet radiation
  - Vacuum ultraviolet radiation
  - Soft x-rays
- Particle radiation exposure
- Temperature cycling
- Atomic oxygen

Results of bend-testing

TABLE 2.—BEND TESTING OF HST SAMPLES

Sample	Surface bent in tension	Cracking occurred	Description of cracks produced/cracking process	Diameter of mandrel to produce first crack or worsening of existing cracks* (mm)	Calculated strain at first crack or first sign of worsening of existing cracks (percent)
SM2 LS-1; cut along curl direction; curl of ~1.5 cm radius	FEP	Yes	Straight/sudden, full-width	9.19	1.93
SM2 LS-3; cut against curl direction	FEP	Yes	Straight/sudden, full width	4.29	2.54
SM2 CVC-1; contains vent cut	FEP	Yes	jagged/gradual	9.19	1.63
SM2 CVC-2; typical region	FEP	Yes	jagged/gradual	7.62	1.96
SM1 MSS-A; 16,670 ESH	FEP	Yes	jagged/gradual	4.09	2.67
SM1 MSS-D; 11,339 ESH	FEP	Yes	jagged/gradual	3.56	3.05
SM2 LS-2; cut along curl direction	Al	No			Tolerant to strain >15.25
SM2 LS-4; cut against curl direction	Al	No			Tolerant to strain >15.25
Pristine MLI cut along machine direction	FEP	No			Tolerant to strain >15.25
Pristine MLI cut against machine direction	FEP	No			Tolerant to strain >15.25

*Because the SM1 MSS-A and MSS-D samples contained surface cracks due to handling upon retrieval from HST, data are for worsening of existing cracks.

Result of tensile testing

TABLE 3.—TENSILE TESTING OF RETRIEVED HST MATERIALS

Sample	Yield Strength (MPa)	Ultimate Tensile Strength (Mpa)	Elongation (percent)
Pristine MLI	13.8 14.3 14.3	24.8 26.5 28.1	340 360 390
SM1 MSS-D	14.3 14.3	15.4 16.6	196 116
SM2 CVC	11.0 15.4 N/A	12.1 16.0 11.0	25 25 15
SM2 LS	N/A N/A	13.2 2.2	0 0

Result of hardness

TABLE 4.— SURFACE MICRO-HARDNESS OF RETRIEVED HST MATERIALS

Sample	Hardness at 1 to 1.5 nm	Hardness at 5 nm	Hardness at 15 nm	Hardness at 50 nm	Hardness at 100 nm	Hardness at 200 nm	Hardness at 500 nm
Pristine FEP/Al	0.27 ± 0.64 at 1 nm	0.09 ± 0.05	0.07 ± 0.01	0.07 ± 0.008	0.06 ± 0.007	0.06 ± 0.004	0.06 ± 0.002
SM1 MSS-G	0.28 ± 0.25 at 1.5 nm	0.21 ± 0.25	0.13 ± 0.03	0.09 ± 0.02	0.08 ± 0.01	0.07 ± 0.01	0.06 ± 0.004
SM1 MSS-B/C	-	-	0.26 ± 0.10	0.15 ± 0.05	0.11 ± 0.03	0.10 ± 0.01	0.08 ± 0.02
SM1 MSS-E/F	-	-	0.31 ± 0.11	0.20 ± 0.07	0.16 ± 0.03	0.11 ± 0.02	0.07 ± 0.01
SM1 MSS-D	-	-	0.34 ± 0.07	0.20 ± 0.05	0.15 ± 0.02	0.11 ± 0.02	0.07 ± 0.009
SM1 MSS-A	-	-	0.44 ± 0.14	0.26 ± 0.10	0.17 ± 0.07	0.12 ± 0.07	0.08 ± 0.05
SM2 CVC	0.35 ± 0.16 at 1 nm	0.24 ± 0.20	0.18 ± 0.10	0.18 ± 0.08	0.13 ± 0.06	0.07 ± 0.05	0.09 ± 0.001
SM2 LS	0.46 ± 0.17 at 1 nm	0.32 ± 0.26	0.14 ± 0.06	0.13 ± 0.02	0.13 ± 0.02	0.13 ± 0.04	0.10 ± 0.01

Results of bend-testing

Scanning electron photomicrograph of cross section of crack surface

Cracked surface