Spacecraft Sterilization using Non-equilibrium Atmospheric Pressure Plasma

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Current Methods used for Spacecraft

According to NASA regulations, landers and probes must have ≤ 30 bacterial spores on the free surfaces of a landed system to prevent the proliferation of Earth's microorganisms. Current methods to achieve spacecraft sterilization have several drawbacks which can be eliminated by employing non-equilibrium atmospheric pressure plasmas.

Procedure	Technique—Problems
Alcohol wipes	**Swabbing-** Interior surfaces (e.g., electronic components) are inaccessible
Dry heat	**105-180 °C for 1 to 300 hrs** - can lead to the failure of electronic components
Wet heat	**120-134 °C for 3 to 20 min-** corrosion and water absorption
B-radiation	**1 to 10 MeV** - Limited penetration
Γ-radiation	**2.5 Mrad-** optical changes in glasses and damage to electronics, solar cells
UV	**5,000 to 20,000 J/m²** - unexposed surfaces remain untreated

Efforts were focused on treatment by direct plasma via dielectric barrier discharge (DBD) , Gliding Arc, and DC glow microdisharge plasma. Experimental evidence of plasma capability of fast and low temperature sterilization of *Escherichia coli*, *Bacillus subtilis* and *Deinococcus radiodurans* were gathered and results show that they have been inactivated and/or completely removed from the surface.

Inactivation Mechanisms

Physical Mechanisms

Global [applied] Effects

Global effects of: a) E-field; b) Temperature
1. **Electroporation**
2. **Surface Heating (cooking)**

Effect of Neutral Species

a) Long-lived (O3, NO, HO2, H2O2)
b) Short-lived (OH,O, elect. excited O2($^1\Delta g$), ...)
1. **Tissue regeneration;**
2. **Natural signal stimulation**
3. **Oxidation**

Ultraviolet (UV) Radiation Effect

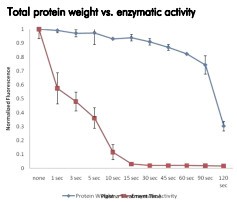
VUV 110-200 nm, UVC 200-290 nm, UVB,A - 290-380 nm
1. **DNA damage**
2. **Cell wall fracture**

Charged Species (Ions & Electrons)

Stimulates oxidation
1.Ion Catalysis
2.Ion Bombardment
3.Collective E-fields
4.Cell wall fracture;
5.Natural signal stimulation
6.Alter protein structure, enzymatic activity; ...

Biological Mechanisms

1. Natural (specific)
   a) Blood Coagulation
   b) Apoptosis initiation (demonstrated with Melanoma cancer cells)
   c) Opening of Calcium ion channels
   d) Direct DNA damage (irreversible)
   e) Protein integrity and enzymatic activity
2. Non-natural (non-specific)
   ~~a) pH~~
   ~~b) Non-equilibrium burning~~



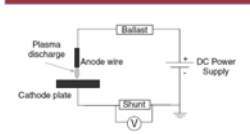
Superoxide Production

The increase in sterilization efficiency when water is added may result from the species formed by the interaction of plasma with water. Plasma induces phospholipid peroxidation thus increasing sterilization efficiency. The plasma-induced formation of superoxides in water is achieved through following set of chemical reactions:

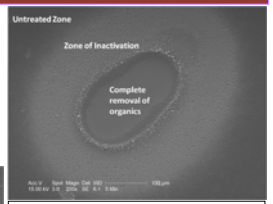
1. $e^-_{(H2O)} + O_{2(H2O)} \rightarrow O_2^-{}_{(H2O)}$
2. $2H^+ + 2O_2^- \rightarrow H_2O_2 + O_2$ (Dismutation reaction)
3. $Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH + OH^-$ (Fenton reaction)
4. $RH + OH \rightarrow H_2O + R^* + O_2 \rightarrow RO_2 + RH \rightarrow RO_2H + R^*$ (Phospholipid peroxidation)
5. $RO_2H \rightarrow RO_2^- + H^+$

The production of hydrogen peroxide and superoxides in the solution will ultimately lead to phospholipid peroxidation, thus aiding in cell death.

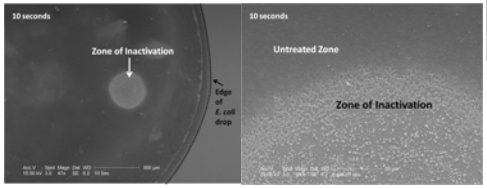
Escherichia coli



DC glow micro-discharge provides higher concentrations of active agents to the targeted area, resulting in a higher efficiency of surface removal capability.
A wire anode approximately 0.5 mm in diameter was used in this setup.

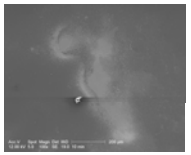


5 minof DC glow discharge treatment.



E. coli on surgical-grade stainless steel after 10 seconds of DC glow discharge treatment at a low (a) and high (b) magnifications.

10 min treatment in Helium with a 1 mm gap between electrode and sample

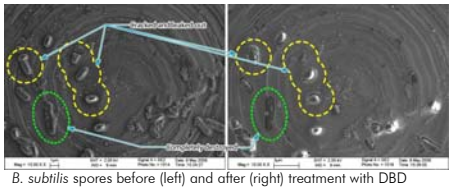


Bacillus subtilis

B. subtilis spores before (left) and after (right) treatment with Gliding Arc plasma (120 sec, 0.8 W/cm2).

- ~$3 \cdot 10^4$ *B. subtilis* per coupon.
- After two minutes of DBD treatment, the spores were rendered unculturable (sample temperature <45°C)
- Similarly, the total number of viable spores per coupon dropped to zero after ten minutes of gliding arc treatment (sample temperature <65°C).

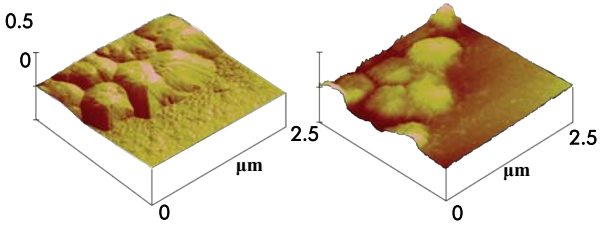
Gliding Arc setup designed for low temperature (45-65 °C) sterilization of metal coupons.



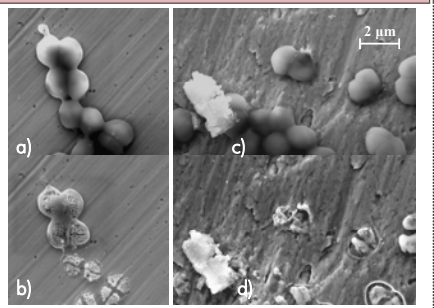
B. subtilis spores before (left) and after (right) treatment with DBD plasma (120 sec, 0.8 Watt/cm2).

Deinococcus radiodurans

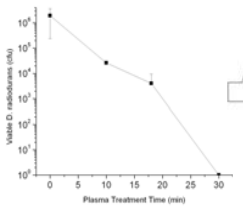
Dry Conditions



Morphological changes before (left column) and after (right column) 10 minutes of DBD treatment.

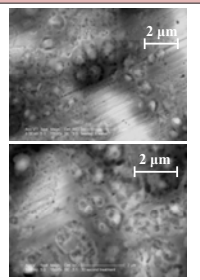


Morphological changes to *D. radiodurans* for 20 minutes (before – a, after – b) and after 30 minutes (before – c, after – d) DBD plasma treatment.

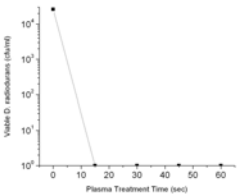


A 6-log reduction of *D. radiodurans* results from 30 minutes of DBD plasma treatment.

Wet Conditions



Morphological changes to *D. radiodurans* in water after 30 sec of DBD treatment.

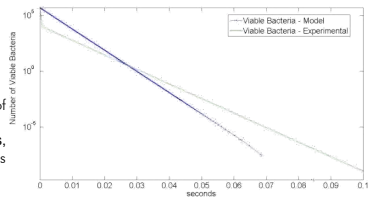


A 4-log reduction of wet *D. radiodurans* results from 15 seconds of DBD plasma treatment.

Survivability as a function of Plasma

$$\frac{d[B]}{dt} = -\kappa_{O_3}[B][O_3] - k_{UV}[B][UV]I - k_{OH}[B][OH] - k_{PI}[B][PI] - k_{e_{aq}}[B][e_{aq}]$$

where [B] is the bacterial concentration, $[O_3]$ is the concentration of ozone, [UV] is the concentration of UV, *I* is UV intensity [μW/cm²], [OH] is the concentration of hydroxyl, [PI] is the concentration of positive ions, $[e_{aq}]$ is the concentration of aqueous electrons, *k* is the reaction rate constant, and *t* is time.



Conclusions

DBD and DC glow microdischarge are effectively inactivates bacteria and spores at low temperatures. A **4-log reduction** of Bacillus subtilis after **DBD treatment for 2 min**, a **4-log reduction** of dry *D. radiodurans* after **DBD treatment for 30 min**, and SEM and AFM images demonstrate that this is an **efficient means of spacecraft sterilization**. Further, it has been shown that treatment by microplasma glow discharge yields **complete removal of organics**. Future research includes sterilization of species in 3D samples, such as dust. With complete removal of protein matter and DNA, the influence of inactivated bacteria will be minimized.

Acknowledgements

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References

Task Group on the Forward Contamination of Europa, Space Studies Board, National Research Council. Preventing the Forward Contamination of Europa. National Academy Press, 2000. p. 18.