Spacecraft Sterilization using Non-equilibrium Atmospheric Pressure Plasma

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Abstract: As a solution to chemically and thermally destructive sterilization methods currently used for spacecraft, non-equilibrium atmospheric pressure plasmas are used to treat surfaces inoculated with *Bacillus subtilis and Deinococcus radiodurans*. Evidence of significant morphological changes and reduction in viability due to plasma exposure will be presented, including a 4-log reduction of *B. subtilis* after 2 minutes of dielectric barrier discharge treatment.

Keywords: Planetary protection, Non-thermal plasma, Sterilization, Disinfection

1. Introduction

It is important that extraplanetary-bound landers and probes have ≤ 30 bacterial spores on the free surfaces of a landed system to prevent the proliferation of microorganisms beyond their original environment [1]. Current methods to achieve sterilization of the exterior surface of the spacecraft have several drawbacks including damage to and failure of electronic components, corrosion, and water absorption. The above problems can be eliminated by employing non-equilibrium atmospheric pressure plasmas. The energized particles, UV radiation, and chemically active species generated in plasma can kill spores, bacteria and other microorganisms at low temperature without thermal and oxidative damage to the treated surfaces.

B. subtilis was chosen for inactivation experiments due to its comprehensive characterization as a model organism for laboratory studies. Due to its highly resistive nature and semblance to "extraterrestrial" bacteria, choosing *Deinococcus radiodurans* is key to replicating a realistic form of bacteria which would return as spacecraft payload.

Evidence of inactivation and/or complete removal of *Bacillus subtilis* and *Deinococcus radiodurans* from the surface have been demonstrated in the presented experiments using dielectric barrier discharge (DBD) plasma and gliding arc (GA) plasma.

2. Methods

Efforts were focused on treatment by direct plasma via dielectric barrier discharge (DBD) (Figure 1a), and gliding arc plasma (Figure 1b).

A standard culturing technique [2-3] was used to follow the survivability of the microorganisms. Samples of *B. subtilis* were prepared on spacecraft materials by depositing 3 x 10^4 *B. subtilis* per 1-cm^2 coupon. The *D. radiodurans* samples were prepared on spacecraft materials by depositing 1.58 x 10^5 *D. radiodurans* per 1-2 cm^2 coupon. Aluminum and stainless steel were two spacecraft materials which were used for these studies. Spacecraft materials were sterilized by ethanol and flaming for 2 minutes prior to the deposition of *B. subtilis* and *D. radiodurans*. 10 drops of 5 µl suspended *B. subtilis* and *D. radiodurans* were dried onto spacecraft materials for 30 minutes in a laminar flow hood.

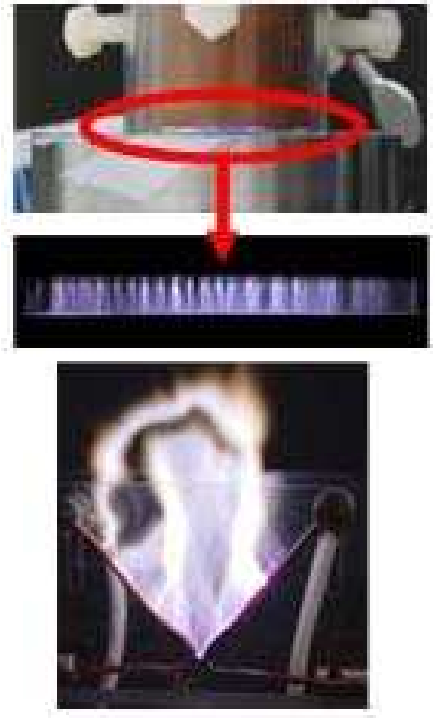


Fig.1 a) Dielectric barrier discharge (DBD) setup and b) Gliding arc (GA) setup.

The coupons were then exposed gliding arc and DBD plasma with a 1 mm gap distance between the sample and the electrode (Figure 2). Experimental evidence of plasma capability of fast and low temperature sterilization of different microorganisms was gathered. Characterization of the destruction phases of *B. subtilis* and *D. radiodurans* during plasma treatment was performed by collecting Scanning Electron Microscope (SEM) images before and after plasma treatment. Cell viability measurements were performed by standard plating method.

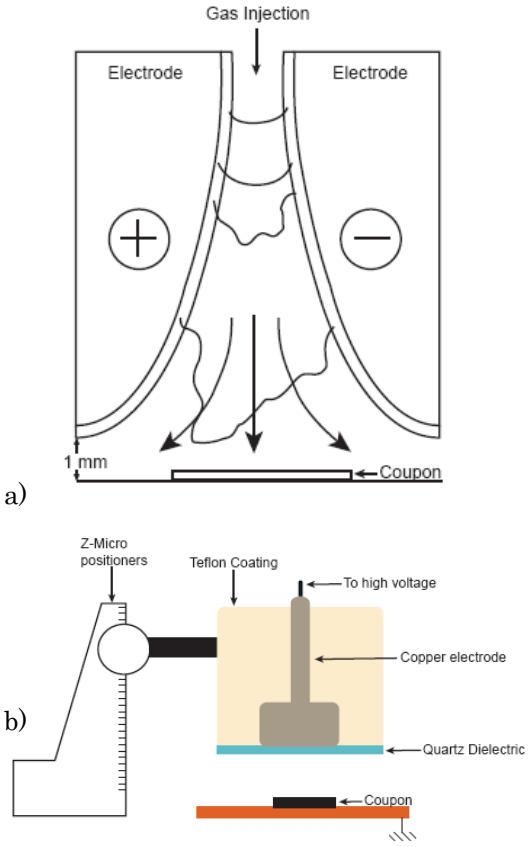


Fig 2. Experimental setup using the a) dielectric barrier discharge (DBD) setup and b) Gliding arc (GA) setup.

3. Results and Discussion

Comparison of Figure 3a (before DBD plasma treatment) and Figure 3b (after 10 min DBD plasma treatment, 45 ºC) clearly shows that DBD plasma causes significant morphological changes of *Bacillus Subtilis* on the sterilization surface at the temperature 45 ºC.

Table 1 shows that complete sterilization of *B. subtilis* was obtained after 2 min of DBD plasma treatment while keeping the coupon temperature at 35-45 ºC. Additionally, complete sterilization was achieved after 10 min GA plasma treatment while coupon temperature remained at 53-65 ºC.

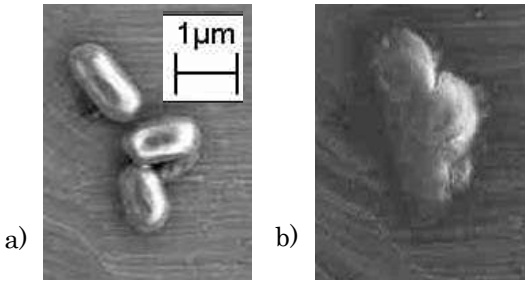


Figure 3. SEM images of *Bacillus Subtilis*.before (a) and after (b) DBD plasma treatment.

Treatment Method	Treatment Time, min	Coupon Temperature, ºC	cfu/ coupon (10 ml)
Pos. Control		r.t.	2.84E+04
Pos. Control		r.t.	3.29E+04
GA	1	42	5.51E+02
GA	10	53	2.00E+00
GA	10	65	0.00E+00
DBD	1	45	1.00E+00
DBD	1	45	1.00E+00
DBD	2	50	0.00E+00
DBD	2	50	0.00E+00
DBD	5	55	0.00E+00
DBD	5	55	0.00E+00
Neg. Control	1	r.t.	0.00E+00
Neg. Control	10	r.t.	0.00E+00

Table 1. Viablity measurements of *B. subtilis* after GA and DBD plasma treatment.

Initial experiments of *D. Radiodurans* inactivation was performed at minimum plasma breakdown power and maximum power for 10 minutes. Table 2 displays the results, showing only a 0.5 log reduction in viability. This result was supported by SEM images taken before and after 10 minutes of DBD treatment at minimum power (Figure 5). There are no morphological changes apparent in the SEM images.

Treatment Time, min	Power	cfu/coupon (10 ml)
0	**-**	**1.2E+06**
10	**Minimum**	**3.4E+05**
0	**-**	**1.2E+06**
10	**Maximum**	**5.4 E+05**

Table 2. Viablity measurements of *D. radiodurans* after DBD plasma treatment.

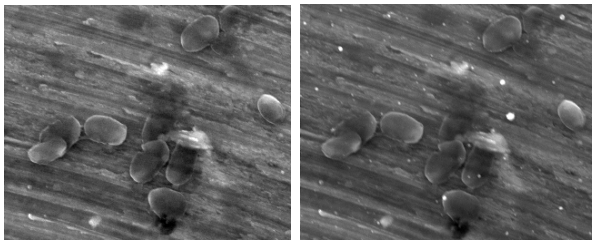


Figure 5. Deinococcus Radiodurans before and after plasma treatment.

Concurrent experiments were performed with twice the power and an increased treatment time of 18 and 30 minutes. One to two-minute breaks were taken per 10 minutes of treatment to prevent the electrode from overheating. The results are shown in Table 3 and plotted in Figure 6**.** After 30 minutes of DBD treatment, there is complete inactivation of *D. radiodurans* with 5-log reduction.

Treatment Time (seconds)	Viable *D. radiodurans* per coupon
0	158000
18	12
30	0.00E+00

Table 3. Viability measurements of *D. Radiodurans* after DBD plasma treatment.

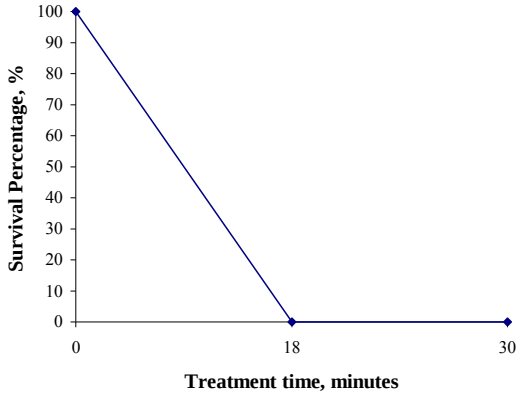


Fig.6 Percentage of viable *D. radiodurans* as a function of DBD treatment time.

Inactivation mechanisms

The inactivation of *B. subtilis* and *D. Radiodurans* can be caused either by an individual or synergistic effects of plasma components interacting with the microorganism. A list of possible inactivation mechanisms by plasma includes [4]:

1. Lysis of the bacterium as a result of the rupture of its membrane due to the electrostatic force exerted on it by accumulation of charged particles coming from the plasma.
2. Lethal damage to the microorganism genetic material by UV irradiation.
3. Erosion of the micro-organisms through intrinsic photodesorption.
4. Erosion of the micro-organisms through etching by reactive species.
5. Diffusion of oxygen atoms and, more generally, of oxygenated species (e.g. OH) through the spore material with resulting local damage possibly by oxidation to the cytoplasmic membrane, proteins and DNA material

Plasma has an added biocidal effect [5] in liquid, as it stimulates the creation of superoxides. In the presence of oxygen, the following set of reactions occur:

1. $e^-_{(H2O)} + O_{2(H2O)} \rightarrow O_2^-{}_{(H2O)}$
2. $2H^+ + 2O_2^- \rightarrow H_2O_2 + O_2$ (dismutation rxn)
3. $Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH + OH^-$
4. $RH + OH \rightarrow H_2O + R^* + O_2 \rightarrow RO_2 + RH \rightarrow RO_2H + R^*$

   $RO_2H \rightarrow RO_2^- + H^+$

Future experiments and simulations will focus on evaluating all contributors, and thus bring forth more understanding on the mechanisms of plasma sterilization.

4. Conclusions

DBD and gliding arc are effective means by which to successfully inactivate bacteria and spores at low temperatures. Viability measurements and SEM images demonstrate that this is an efficient means of spacecraft sterilization. Future experiments will expound on the degree of death undergone by the bacteria through polymerase chain reaction (PCR) measurements.

Acknowledgement

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