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Cold atmospheric plasma – A new technology for spacecraft component decontamination

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ABSTRACT

Cold atmospheric plasma (CAP) based on the Surface Micro-Discharge (SMD) technology was investigated for inactivation of different bacteria and endospores. The used technique was developed to serve as an alternative method for the decontamination of spacecraft components based on the COSPAR planetary protection policy where currently the dry heat microbial reduction method is the only applicable way to satisfy the required demands. However it is known, that dry heat can thermally damage sophisticated components installed on the device. Therefore, the development of a low temperature sterilization system is one of the high priority issues for upcoming space missions in the extraterrestrial field. In the study presented here, the vegetative bacteria *Escherichia coli* and *Deinococcus radiodurans* and several types of bacterial endospores – including *Bacillus atrophaeus*, *Bacillus safensis, Bacillus megaterium, Bacillus megaterium* 2*c*1 and *Bacillus thuringiensis E*24 – were inactivated by exposing them indirectly i.e. only to the reactive gases produced by the SMD electrode at room temperature. The results showed a 5 log inactivation for *E. coli* after 10 min of exposure. In contrast *D. radiodurans* proved to be more resistant resulting in a reduction of 3 log after exposure of 30 min. More than 6 log reductions were achieved for *B. safensis, B. megaterium* and *B. megaterium* 2*c*1 after 90 min of exposure. Furthermore the applicability of the used CAP system for spacecraft decontamination according to the planetary protection policy was investigated. This included also the investigation of the inactivation homogeneity by the plasma gas, the control of the temperature at the area of interest, the measurement of the O_3 density in the treatment region and the detailed investigation of the effects of the exposure on different materials.

1. Introduction

Astrobiology is an emerging area of research aiming at the study of the origin, evolution and distribution of life within the context of cosmic evolution. The main scientific question is whether the emergence of life is an inevitable event when suitable environmental conditions exist or not (de Duve, 2011). The search for extraterrestrial life is therefore an important goal of ongoing and scheduled space exploration missions. The only region in space, that is currently accessible to direct *in situ* investigation, is our solar system. Manned and robotic missions to the Moon, to asteroids, to the outer planets and their moons will all be governed by the quaestio cardinalis whether life did or does exist on other bodies in our solar system. Robotic missions, e.g. Viking, Cassini-Huygens or Phoenix Lander, Mars Express, Mars Reconnaissance Orbiter, the MER rovers Spirit and Opportunity and the MSL rover Curiosity on Mars have already gathered a wealth of data which has broadened our perspective with regards to search at favorable places for traces of organics, water and possibly life or fossil fragments thereof (Blumberg, 2011).

All challenging and costly missions of the near future bear a common risk. One could easily obtain false positive answers to the question of the existence of life, due to a contamination of the

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spacecraft or parts of it, by an earthly organism, a kind of stowaway. Already in 1967, Article IX of the *UN Treaty on Principles Governing the Activities of States in the Exploration and Use of Outer Space, including the Moon and Other Celestial Bodies* addressed the possible risk of planetary contamination. The United Nations mandated COSPAR, the Committee on Space Research, to develop, maintain and promulgate a planetary protection policy "for the reference of spacefaring nations, both as an international standard on procedures to avoid organic constituent and biological contamination in space exploration, and to provide accepted guidelines in this area to guide compliance with the wording of this UN Space Treaty and other relevant international agreements". The definition for maximal bioburden limits depends on mission target, type of mission and aim of the mission. For instance, total bioburden level should be maintained below 5×10^5 spores in the case of no direct contact missions such as flyby (category III), and it should be below 3×10^5 spores with maintaining the surface density below 300 spores/m^2 in the case of direct contact missions such as the one with landers, probes and some orbiters (category IV). This policy is updated on a regular basis according to the actual state of knowledge. Planetary protection in this sense is the term used to describe guiding principles in design of an interplanetary mission that aims to prevent biological contamination of both the target celestial body (forward contamination) and the Earth (backward contamination). This principle arises from the scientific need to preserve planetary conditions for future biological and organic constituent exploration and to protect the Earth–Moon system from potentially harmful extraterrestrial agents.

The assembly, integration and test of spacecraft hardware in bioburden controlled cleanrooms and the application of different microbial reduction methods are used to accomplish microbial control when this is necessary for a specific mission.

Bioburden reduction processes can be divided into physical, chemical or mechanical methods, e.g. sterilization by dry or wet heat, by ionizing or non-ionizing radiation, by exposure to chemically reactive gases or liquids or by filtration. Each of these methods has advantages and limitations which have to be considered before choosing a method for a specific purpose. For planetary protection ESA and NASA currently have only one approved method for spacecraft sterilization – the dry heat microbial reduction (DHMR) process. This technique was used on the Viking Mars landers, which were built and launched in the 1970s. However, advanced materials, electronics, and other heat-sensitive equipment used on spacecraft today could be damaged by such high-temperature treatment. Therefore, both space agencies are currently developing and standardizing alternative sterilization methods for application on spacecraft components and systems. These methods have to fulfill the general requirements for new bioburden reduction methods which imply the following: they should be effective in biological inactivation where bacterial endospores are the main target, applicable to different types of spacecraft materials and components, the required treatment time should be short and the method should not damage materials.

Due to their unique properties, bacterial endospores, e.g., from *Bacillus* and *Clostridia* spp., are known to be some of the most resistant terrestrial microorganisms. Spores are dormant without possessing any metabolic or reproductive activity. They have a robust structure with several dense spore coat layers (cortex, inner/outer spore membranes and coats) which surround the important DNA in the core (Driks, 1999; Henriques and Moran, 2007; Legett et al., 2012). Furthermore the DNA is additionally protected by small-acid soluble proteins (Setlow, 2007). Spores withstand inactivation by wet heat due to the very low water, high dipicolinic acid and mineral content of their cores (Legett et al., 2012). These properties are the main reasons, why spores are able to endure harsh environments with high temperature changes, oxidative stress, irradiation, desiccation etc. (Setlow, 2006). Under favorable conditions with sufficient nutritional supply, spores leave the dormant state and return to the vegetative form where they start to grow again. Due to the above mentioned reasons endospores are used as model microorganisms for testing different sterilization methods just as autoclaves, H_2O_2 gas sterilization, gamma-irradiation etc.

As a new medium cold atmospheric plasma (CAP) has been widely investigated for several – mainly biomedical – applications (Laroussi, 2002; Stoffels, 2007; Fridman et al., 2008; Stoffels et al., 2008; Kong et al., 2009; Morfill et al., 2009a; Morfill et al., 2009b; Weltmann et al., 2010). This included the design and development of different CAP devices possessing different characteristics, i.e. produced reactive species, composition and concentration of species (Ehlbeck et al., 2011), which achieved effects such as sterilization/disinfection (Klaempfl et al., 2012), blood coagulation (Kalghatgi et al., 2007), cell proliferation (Nosenko et al., 2009), cancer cell inhibition (Keidar et al., 2011).

One important aspect of CAPs is that the temperature of the gas which reaches the target is below 40 °C. This low temperature allows the treatment of heat sensitive materials including for example human skin (Isbary et al., 2010), without thermal damage. CAPs are furthermore gaseous, which allows a non-contact treatment. In addition, due to the mobility of the gas, the treatment into tiny pores is also feasible.

CAPs generate a reactive mix composed of reactive nitrogen and oxygen species (RONS), UV photons, charged particles and electric fields due to local charging. All these species are produced in the plasma as well as during the transport from the electrode itself to the target.

The interaction between CAP species and the target sites (e.g. pathogens, eukaryotic cells, tissue, different materials) depend on the plasma production method as well as on the configuration of the device and resulting gas composition. For instance, when using plasma jets (Walsh et al., 2006) or Floating-Electrode Dielectric Barrier Discharges (Fridman et al., 2006), the plasma discharge gets in direct contact with the target, so that all produced components are involved in the interaction reactions. In contrast, when using Surface Micro-Discharge (SMD) plasma devices, the plasma itself is restricted to a very thin layer near the electrode, and only long-lived reactive species interact with the target (Morfill et al., 2009a).

In 2008 and 2009, the studies on planetary protection using CAPs were conducted (Schuerger et al., 2008; Cooper, 2009; Cooper et al., 2009). In those studies inactivation of *Bacillus subtilis*, which is one of the common spacecraft contaminants, prepared on several types of spacecraft materials (Schuerger et al., 2008), and *Bacillus stratosphericus* and *Bacillus pumilus* spores, which are known to be resistant against UV radiation and therefore represent a problem for space missions were treated with different Dielectric Barrier Discharge (DBD) plasma configurations (Cooper, 2009; Cooper et al., 2009). Whereas the DNA of the spores suspended in liquid was disintegrated within 60 s of the CAP treatment and etching occurred on dry spore samples, the inactivation of the extreme environmental resistant *D. radiodurans* placed on dry stainless steel substrates was achieved only after 30 min of CAP treatment. Inactivation of spores using DBD is also well reviewed by Boudam et al. (2006) and Ehlbeck et al. (2010, 2011). Palagonite burden (Martian soil with various metal oxides) demonstrated an enhanced inactivation of spores (Cooper, 2009). Similarly, Jung et al. (2010) observed an improved inactivation by the use of TiO_2 catalyzer during the plasma process.

However, most setups of the aforementioned CAP systems, where it is necessary to put a treatment target on the site facing to a plasma discharge, are not appropriate for the decontamination of