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In package inactivation of *Bacillus atrophaeus* spores using high voltage atmospheric cold plasma

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ABSTRACT

Introduction: Hospital acquired infections are of a great concern, considering a large number of infections reported every year. Sterilization is an important step in healthcare industry that is attained by utilizing conventional sterilization approaches. It includes heat treatment, use of chemicals like ethylene oxide, hydrogen peroxide, and gamma radiation. These methods have drawbacks such as material properties of medical devices could be altered or damaged. Therefore, it is necessary to investigate cheap alternative approaches to achieve sterilization without generating toxic residues. Nonthermal atmospheric plasma is a fourth state of matter that consists of charged particles, positive and negative ions and number of reactive species. This plasma mixture has greater microbicidal effects on number of food products and wide range of surfaces. Novel nonthermal plasma technology has number of applications in food and medical industries.

Methods: The objective of this study was to investigate the effect of plasma parameters on inactivation of resistant biological forms of *Bacillus atrophaeus* inside a sealed package. *Bacillus atrophaeus* spore strip (spore population 6.36 log10/strip) was placed in a petri dish, sealed in a polypropylene container, and was subjected to high voltage atmospheric cold plasma treatment (HVACP). HVACP system was operated at 70 kVRMS and at a frequency of 50 Hz. The two 15-cm diameter aluminum disk electrodes were separated by a rigid polypropylene container which served as a sample holder and as a dielectric barrier. The distance between the two electrodes was equal to the height of the container (22 mm). The top electrode served as a high voltage electrode and bottom electrode was grounded. The discharge was monitored using electrical probes and an Agilent InfiniVision 2000 X-Series Oscilloscope. Influence of different process parameters on spore inactivation including treatment time, mode of exposure (direct/indirect), and working gas types were mainly evaluated. Effect of relative humidity on HVACP inactivation efficacy was also assessed. The inactivation efficacy was determined using standard colony count method. To assess gas composition following HVACP exposure, optical absorption spectroscopy was used.

Results: A strong effect of process parameters on inactivation was observed. Direct exposure to plasma was very effective for spore inactivation, achieving ≥6 log cycle reduction of spores in all gas types tested, in only 60 s of treatment time. However, a strong influence of gas type was noted on spore reductions where indirect mode of plasma exposure was utilized. The relative humidity also noted as a critical factor in bacterial spore inactivation by HVACP, where a major role of plasma generated species other than ozone was noted.

Conclusion: Overall, a strong influence of process parameters on spore inactivation was noted. Effective in-package bacterial spore inactivation within 30-60 s demonstrates the promising potential application of HVACP for sterilization of medical devices and heat sensitive materials.