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Influence of high voltage atmospheric cold plasma process parameters and role of relative humidity on inactivation of *Bacillus atrophaeus* spores inside a sealed package

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SUMMARY

Background: Non-thermal plasma has received much attention for elimination of microbial contamination from a range of surfaces.
Aim: This study aimed to determine the effect of a range of dielectric barrier discharge high voltage atmospheric cold plasma (HVACP) parameters for inactivation of *Bacillus atrophaeus* spores inside a sealed package.
Methods: A sterile polystyrene Petri dish containing *B. atrophaeus* spore strip (spore population 2.3×10^6/strip i.e. 6.36 $\log_{10}$/strip) was placed in a sealed polypropylene container and was subjected to HVACP treatment. The HVACP discharge was generated between two aluminium plate electrodes using a high voltage of 70 kV_{RMS}. The effects of process parameters, including treatment time, mode of exposure (direct/indirect), and working gas types, were evaluated. The influence of relative humidity on HVACP inactivation efficacy was also assessed. The inactivation efficacy was evaluated using colony counts. Optical absorption spectroscopy (OAS) was used to assess gas composition following HVACP exposure.
Findings: A strong effect of process parameters on inactivation was observed. Direct plasma exposure for 60 s resulted in $\geq$6 $\log_{10}$ cycle reduction of spores in all gas types tested. However, indirect exposure for 60 s resulted in either 2.1 or 6.3 $\log_{10}$ cycle reduction of spores depending on gas types used for HVACP generation. The relative humidity (RH) was a critical factor in bacterial spore inactivation by HVACP, where a major role of plasma-generated species other than ozone was noted. Direct and indirect HVACP exposure for 60 s at 70% RH recorded 6.3 and 5.7 $\log_{10}$ cycle reduction of spores, respectively.

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

Introduction

Non-thermal atmospheric plasma is gaining interest for a number of applications including decontamination of contaminated surfaces, improvement of food safety, material surface treatment, and sterilization of medical instruments.[1–3]

Plasma discharge results in generation of a number of antimicrobial agents including reactive oxygen species (ROS), reactive nitrogen species (RNS), ultraviolet (UV) radiation, energetic ions, and charged particles. Parameters such as applied voltage, mode of plasma exposure, gas type, treatment time, and relative humidity (RH) influence the generation of reactive species, thus affecting the overall process. The type of reactive species and their concentration depend on the type of gas used for plasma discharge, thereby dictating the microbial inactivation efficacy.[4,5] The role of various charged particles and reactive species generated in the mechanism of plasma inactivation is under investigation.

Plasma treatment for the inactivation of spores using low pressure, atmospheric pressure and surface micro-discharge-type plasma has been reported.[6–8] The significant role played by relative humidity on the plasma inactivation of spores has also been investigated in previous works.[5,9] The influence of increasing RH level on ozone germicidal efficiency and the likely contribution of additional radicals such as hydroxyl ion and peroxides in the inactivation process have been reported.[10,11]

In the current work, in-pack HVACP inactivation of *B. atrophaeus* spores using a high voltage level over short exposure times (30–120 s) in a sealed environment was evaluated for the first time. The effect of mode of exposure and process parameters including treatment time, gas type, and the interactive effect of RH on inactivation of spores were investigated. The inactivation of spores is explained and correlated to the plasma-induced gas chemistry and the generation of highly oxidizing species in a sealed package. System diagnostics included optical absorption measurements which were collected under identical experimental conditions.

Methods

HVACP system set-up

The HVACP device (Figure 1) is an atmospheric low temperature plasma generator. The HVACP system was operated at 70 kV_{RMS} at a frequency of 50 Hz. The two 15 cm diameter aluminium disc electrodes were separated by a rigid polypropylene container (310 × 230 × 22 mm) which served as a sample holder and as a dielectric barrier with wall thickness of 1.2 mm. The distance between the two electrodes was 22 mm, equal to the height of the container. The top electrode served as a high voltage electrode and the bottom electrode was grounded. The discharge was monitored using electrical probes [1:1000 voltage probes PV6 (North Star, Tucson, AZ, USA) and a current probe CT-E1.0S (Bergoz Instrumentation, Saint-Genis-Pouilly, France)] and an Agilent InfiniVision 2000 X-Series Oscilloscope (Agilent Technologies Inc., Santa Clara, CA, USA). The HVACP obtained at 70 kV_{RMS} and about 100 kV peak amplitude over large gaps (20 mm) works in the filamentary mode with transferred power in the range of 40 W and discharge duty cycles of 0.5 corresponding to discharge on-times of 5 ms. The analysis is based on capacitance measurements or Manley's method.[12,13]

HVACP treatment

Gas types

Three gas types were used for HVACP generation, namely atmospheric air (gas type 1), 90% N_2 + 10% O_2 (gas type 2), and 65% O_2 + 30% CO_2 + 5% N_2 (gas type 3). The specified concentration gas types were purchased from BOC, Dublin, Ireland.

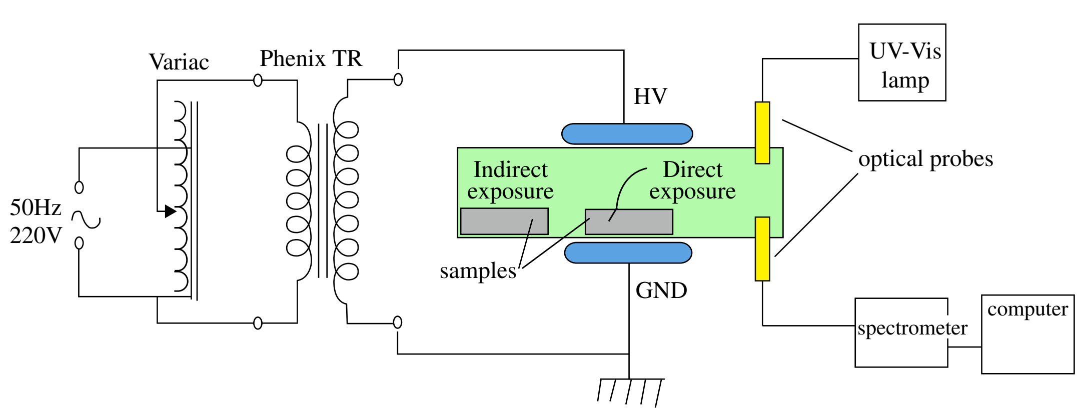

Figure 1. Schematic representation of high voltage atmospheric cold plasma (HVACP) device. TR, transformer; HV, high voltage; GND, grounded; UV-Vis, ultraviolet-visible spectrum.