Investigation of bacterial spore inactivation using a 2.45 GHz coaxial plasma source

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The aim of this work was to assess the influence of gas pressure on the inactivation kinetics and cell morphology of *B. atrophaeus* spores exposed to the plasma generated by a low pressure plasma source. A coaxial electron cyclotron resonance (ECR) source was used to generate air plasma that was used for the treatment of surfaces artificially contaminated with endospores. A typical two-phase inactivation kinetics, with a fast spores reduction (2-log in 15 seconds) followed by a slow rate reduction (around 5-log reduction in 10 minutes) was observed for the plasma treatment at different pressures. In the same time, the preliminary results suggest that the gas pressure does not influence the inactivation of *B. atrophaeus* spores.

1. Introduction

One of the main concerns in the food industry is the life-self of products for which the treatment with the conventional preservation techniques such as heat treatment influence the food quality [1]. In this context, cold plasma treatment appears as a highly promising alternative technique for food preservation [2-3]. However, the high number of parameters that affect the plasma characteristics hinders the understanding of the mechanisms involved in the microorganisms' inactivation.

The present study investigates the influence of gas pressure on the inactivation of *Bacillus atrophaeus* spores in plasma generated by a coaxial electron cyclotron resonance (ECR) source.

2. Material and methods

2.1. Experimental set-up

The plasma source evaluated for its biocidal effect was a 2.45 GHz coaxial ECR plasma source called *Aura source*™ (SAIREM SA). The source was placed on the upper side of a vacuum chamber and was water cooled as shown in Figure 1. Bio-samples may be placed at four different heights *h* from the plasma source.

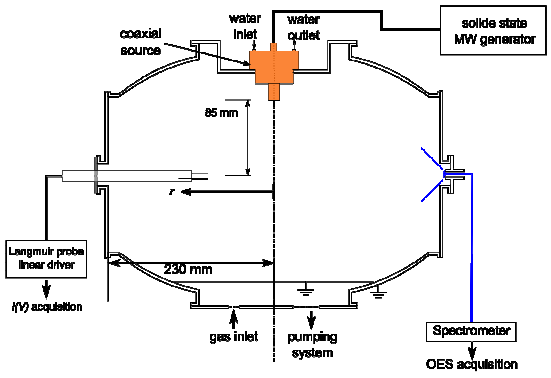

Figure 1: Schematic of experimental setup

Prior to any treatment of biological samples, the vacuum chamber was pumped down to 10^{-4} mbar base pressure introducing a permanent air flow in order to maintain the chamber at a targeted pressure in the 10^{-2} mbar range. The plasma source was connected to a 2.45 GHz solid state generator which provided up to 200 W input power at the electron cyclotron resonance frequency.

2.2. Plasma characterization

For the assessment of the relationship between the biocidal efficiency and the plasma characteristics, a Langmuir probe (Impedans Ltd.) and a 200–1100 nm spectrometer (QP600-2-SR Oceano Optics) were used to extract plasma parameters. Diagnostics were performed at mid-height from the plasma source. While the probe was linearly driven with a step motor allowing radial resolution of probe measurements, the spectroscopy measurements integrate the plasma emission from the whole chamber.

2.3. Sample preparation and assessment of spores' viability after plasma exposure

A stock of *B. atrophaeus* NRRL B4418 containing approximately 10^8 spores/ml was used to inoculate sterile 20 mm x 20 mm glass plates. 10 µl of spore suspension was spotted in the centre of the glass carriers and left to dry at room temperature in a laminar flow hood. After drying, the samples were placed in the centre of the chamber (95 mm from the plasma source) and exposed individually to the air plasma at different pressures. The exposure time varied from 15 to 600 seconds. For the assessment of spores' viability spatial distribution after plasma treatment, the samples were exposed for 60s in different positions of the reactor chamber. After the treatment the spores were recovered in phosphate

buffer containing 0.1% Tween 80. Viable spore counts were performed by serial dilutions on Tryptic soy agar plates after 24 h of incubation at 35°C.

2.4. SEM imaging

Isopore membranes (13 mm diameter, Milipore) were inoculated with *B. atrophaeus* spores, dried and exposed to the plasma emission as described previously. Treated and untreated samples were Pt plated before observation by scanning electron microscopy using an ESEM Quanta 250 (FEI, Oregon, USA) equipped with a Schottky field emission gun (FEG) for optimal spatial resolution.

3. Results and discussion

3.1. Plasma characteristics

Typical plasma optical emission obtained is shown in Figure 2. For each measurement, identified emission intensities susceptible to indicate the presence of reactive nitrogen and oxygen species may be extracted and plotted as a function of pressure and other plasma operating parameters.

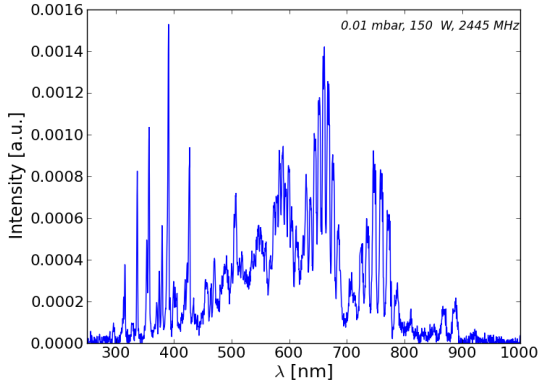

Figure 2: Optical emission spectrum at 1 Pa and 150 W

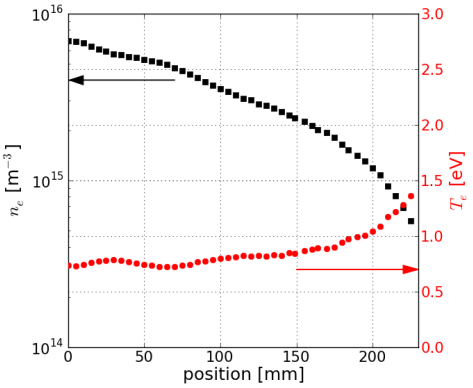

Figure 3: Electronic density (■) and temperature (●) spatial distribution at 1 Pa and 150 W

Typical plasma density and temperature radial profiles obtained at 1 Pa are shown in Figure 3. Plasma characteristics, also measured in air at 5 Pa for comparison purpose, will be presented and discussed.

3.2. Inactivation kinetics of *B. atrophaeus* spores

The spores' inactivation followed a two-phase pattern with a first phase (from 0 s to 15 s) characterized by an abrupt decrease in the surviving number of spores (-2 log) and a second phase having a reduced spores inactivation rate (Fig. 4). For the studied pressures (1 Pa and 5 Pa), 2-log reduction and $\geq$ 5-log reduction was observed after the exposure to the plasma for 15 seconds and 10 minutes respectively, suggesting that the pressure does not have a substantial effect on the inactivation kinetics of *B. atrophaeus* spores. Nevertheless, a complete study of the inactivation kinetics for plasma treatments operating at other gas pressures are needed to confirm these data.

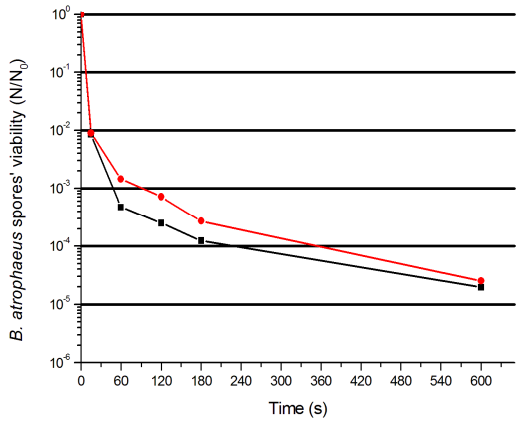

Figure 4: Survival curves of *B. atrophaeus* spores after plasma exposure to the air plasma at 1Pa (■) and 5 Pa (●)

In order to obtain a 2D mapping of the biocidal efficacy, the number of surviving spores after plasma treatment will be also plotted against the positions of the sample in the reaction chamber. Furthermore, the spatial distribution of biocidal effect will be compared with the plasma spatial profile.

3.2. Effect of the plasma treatment on spores' morphology

The structure and dimensions of plasma exposed spores were compared with untreated spores using scanning electron microscopy (Fig 5). The untreated samples (Fig.5.A.) contained rod-shaped spores with relatively homogeneous size and shape. The exposure to the plasma emission generated extensive modifications of the spores' external morphology with the loss of the spores' coat integrity and

leakage of the internal content (Fig. 5.B.). Similar morphological changes have been reported for the exposure of *Bacillus* spores to low pressure Ar plasma [4] and the authors implied that the observed erosion is effect of the ions and reactive species present in the plasma.

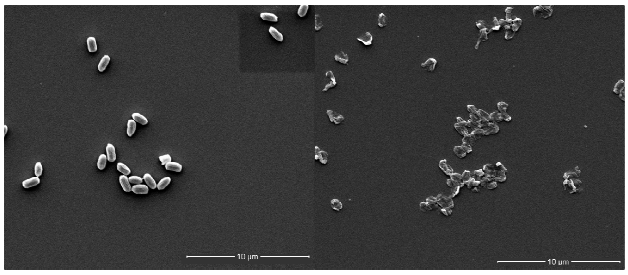

Figure 5: SEM micrographs of *B. atrophaeus* spores. A. Untreated sample; B. Plasma treated sample (5 minutes; 1 Pa; 150 W)

SEM images were also used to assess the relationship between the number of surviving microorganisms following the plasma treatment and the change in the spores' morphology.

4. Conclusions

We have investigated the effect of the gas pressure on the spores' inactivation using a 2.45 GHz coaxial ECR plasma source. Our preliminary results suggest that the pattern of inactivation kinetics, which appears as a two-phase event, is not influenced by the gas pressure. SEM imaging was used to evaluate the effect of plasma treatment on the cell morphology Extensive morphological changes following the plasma treatment were observed. A complete study of the inactivation kinetics for plasma treatments operating at several gas pressures the will be presented. In order to evaluate the uniformity of the plasma treatment, the decontamination results obtained for several positions will be used to determine the 2D distribution of the biocidal efficiency for different pressures.

4. References

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