Diagnostic of reactive species in a dielectric barrier discharge air/helium plasma for the treatment of a pre-packed food simulant

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I. Introduction

There is a need for decontamination and sterilization of food produce on a large scale, using minimally invasive technologies which do not alter the appearance or taste of the produce. Treatment with atmospheric plasma generated in a dielectric barrier discharge (DBD) is currently investigated as one promising technology achieving these aims [1]. However, the detailed physical/chemical mechanisms generated in the plasma which leads to microbial inactivation are not fully understood yet [2].

For example, Moisan et al. [3] have described both damage and etching of the bacterial cells by ultraviolet light and the action of atomic and molecular radicals produced by the plasma. Cellular damage caused by plasma-generated electrostatic stress [4] and cell wall damage caused by ion perforation and ozonation [5] have also been reported. The main radicals and reactive species identified in these processes are atomic oxygen, ozone, OH, NO and NO_2. DBD plasmas are efficient at producing ozone from pure oxygen gas [6]. This feature has been used in the PK-1 device [1] which has potential industrial application. The objectives of the present research were, thus, to (1) implement plasma diagnostics to characterize this device (2) investigate the contribution of residual ozone and radicals to microbial inactivation on a food simulant for a period up to 24 hours after treatment with the PK-1 device .

II. Experimental Details

Our apparatus consisted of a transformer converting the mains voltage and frequency to 15 kV and 60 Hz with a maximum current rating of 30 mA. The transformer was operated at an output power of 40 W. The electrodes connected to this transformer were made of high voltage copper cable coiled around dielectric cardboard bases of dimensions 8.5 cm × 6 cm.

A polyethylene zip lock bag containing a gas at atmospheric pressure was sandwiched in between these two electrodes. The bag was filled with a helium-rich air-helium mixture. Gas was allowed to escape until the desired electrode gap was achieved, ranging between 3.5 cm and 15 cm. Frame images of the luminous plasma structure were captured with a 80 mm focal length convex lens focused onto an intensified CCD array. Each frame was integrated for a period of 35 ms. The images are displayed on false-colour intensity maps. The plasma emission was captured via a 0.22 NA optical fibre and analyzed using a low-resolution UV-VIS spectrometer operated in the wavelength range 200 nm – 1,000 nm. The optical fibre was aligned at the centre of the plasma at a distance of 6 cm.

Listeria innocua NCTC 11288 was the non-pathogenic strain used for this study. Cells were grown in 10mL of TSB-G, incubated for 18 hours, harvested by centrifugation at 10,700 rpm for 10min at 4 ºC and washed with sterile phosphate buffered saline. A model system of agar-agar gel was used to assess the efficacy of plasma treatments on microbial inactivation. 1mL of the washed culture was spread onto each of the agar-agar plates. After a drying period of approximately 30 minutes the plates (without the lid) were placed into the bags previously mentioned and then between the two electrodes, ensuring that the petri dish was not in the direct path of the plasma stream. A 5 cm electrode gap was used during the plasma processing. The treatment was carried out for 5 minutes at 22^{o}C. Each sealed bag was then stored at room temperature for 1.5, 3, 18, 21 and 24 hours, respectively. Experiments were conducted in triplicate i.e., three separate plasma treatments of the same prepared inoculum. Colony forming units (CFU per cm^2) were obtained after (i) stomaching of the (treated) agar-agar samples in Maximum Recovery Diluent (MRD), (ii) performing appropriate dilutions in MRD, (iii) plating 0.1 mL of the diluted sample in TSA plates and (iv) incubating the plates at 37^{o}C for 48 hours.

III. Results and discussions

Typical 35 ms frame images of the plasma are presented in Figs 1(a,b,c). At an electrode separation of 3.5 cm, the plasma exhibits a mostly homogeneous structure as seen through the roughly uniform emission intensity across its volume, see Fig. 1(a). At electrode separations greater than 6 cm, the plasma structure becomes inhomogeneous, forming long, filament-like "streamers" between the coil electrodes. The emitted light intensity is greatest in proximity to the coils and the least in the centre of the bag but uniform along directions parrallel to the electrodes' surface as seen on Fig1(b). Fig 1(c) shows the highly inhomogeneous structure

obtained when an apple is placed in the middle of the plasma. The apple was supported by a section of a hollow PVC cylinder.

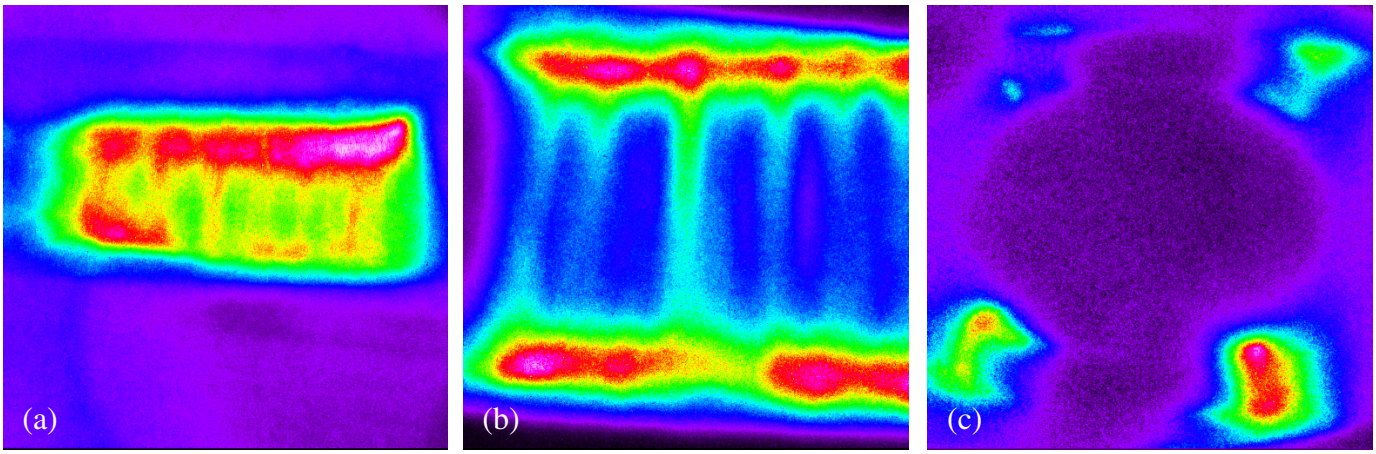

Figure 1. ICCD images of air/helium plasma in a dielectric barrier discharge: (a) 3.5 cm electrode separation, (b) 6 cm electrode separation, (3) with an apple in the discharge volume.

A sample spectrum of the plasma obtained for a 6 cm electrode gap, ie the streaming regime is displayed on Fig. 2. The spectrum is composed of an entirely discrete structure comprising nine intense lines in the wavelength range 320 nm to 400 nm. No continuum emission was observed in the 200 nm – 1,000 nm and the plasma had no visible emission above 400 nm. The spectral features observed can be partly attributed to emission bands in the nitrogen molecule (2^{nd} positive system) and the nitrogen molecular ion N_2^+ (1^{st} negative system). Strong emission lines from various excited states of the atomic species O, O^+, N and N^+ are also found in the same spectral range [7]. These may also contribute to the observed spectrum, however, studies at higher resolution would be required to answer this point. We note the similarity of the spectrum presented in Fig. 2 and with that presented in Fig.6 of Ragni et al. [8] studying surface decontamination of egg shells with a DBD plasma.

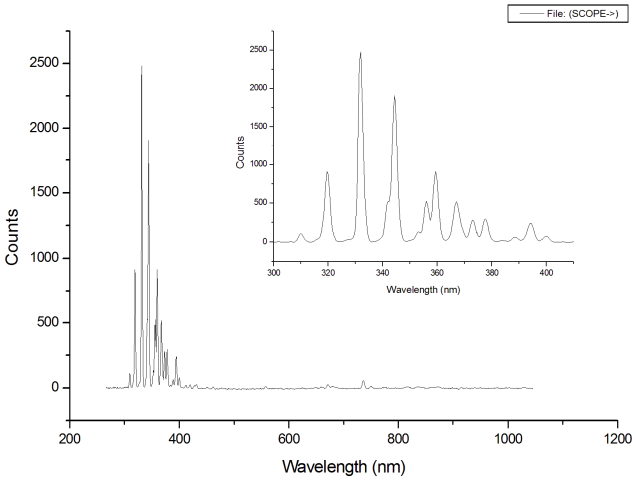

Figure 2. Optical emission spectrum of air/helium DBD plasma measured under low resolution conditions.

The graph of Fig.3 demonstrates that the population of *L. innocua* was significantly reduced after this type of plasma treatment. This reduction was about 2 log cycles with the standard deviation for each point not exceeding 0.5. This inactivation mostly occurred during the

initial 1.5 hours and its rate was significantly reduced thereafter. Possible reasons for this could be the transient nature of the levels of active species (ozone or radicals) which were shown to have short or decreasing half-life cycles [1,2]. Our data cannot ascertain the role played by other plasma antibacterial mechanisms such as electrostatic stress [4] or ion perforation [5].

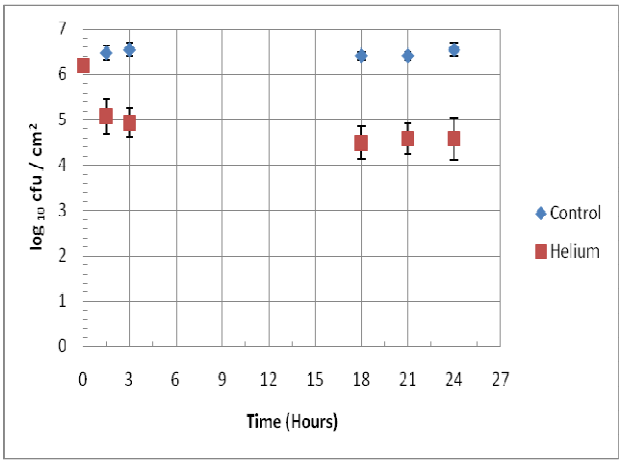

Figure 3. Log cfu/cm² of Listeria innocua inoculated on a food simulant of a non-treated (Control) and post treated in DBD plasma (Helium) for a period of 5 minutes.

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