Deactivation of *Escherichia coli* in a post-discharge chamber coupled to an atmospheric pressure multi-electrode DBD plasma source

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**Abstract.** Experimental results from applying a room pressure RF multi-electrode DBD plasma source to the inhibition of the population growth of Gram negative *Escherichia coli* (*E. coli*) within a post-discharge reactor are reported. The sample to be treated is deposited in the post-discharge chamber at about 50 mm from the plasma source outlet. Thus, the active species generated by the source are conveyed toward the chamber by the working gas flow. The plasma characterization included the measurement of the axial temperature at different distances from the reactor outlet by means of a K-type thermocouple. The resulting 294 K to 322 K temperature interval corresponded to distances between 10 mm to 1 mm respectively. As the material under treatment is placed further away, any thermal damage of the sample by the plasma is prevented. The measurement and optimization of the ozone O₃ concentration has also been carried out, provided that this is an active specie with particularly high germicide power. The effectiveness treatment of the *E. coli* bacteria growth inhibition by the proposed plasma source reached 99% when a 10⁵ CFU/mL concentration on an agar plate had been exposed during ten minutes.

**1. Introduction**
Sterilization by means of a room pressure glow plasma discharge is considered a highly effective method given its multiple advantages. Among them, one finds: the short duration required by its application, its great accessibility to almost any surface topology, as well as its target friendly operation temperature despite its microbial lethality [1].

Several studies have shown that room temperature RF plasmas are generally constituted by ions, free electrons, neutral particles as well as multiple reactive species such as molecular or atomic radicals. It has been long known that the reactive oxygen species (ROS) play an important role in microbial elimination [2]. Thus, in order to enhance the production of ROS such as ozone O₃, which actively interferes with the normal cell respiration process, some proportion of O₂ is added to the discharge working gas [3].

In the relative literature, the concept of bioburden refers to the relative number of viable individuals to be found at any time on the surface of an object or medium [4]. In the particular case of sterilized
surgical instruments, the bioburden oscillates typically within $10^1$-$10^4$ UFC, once the instrument has been used. Furthermore, the bioburden levels after washing remain under $10^2$ UFC [4, 5]. The present study describes the capacity of a room pressure non thermal glow plasma discharge system to inhibit the growth of *E. coli* bacteria cultured on Petri dishes. This, with a view to decontaminating surfaces and, in due course, sterilizing surgical implements.

2. Experimental setup

Figure 1 displays the experimental array assembled around a portable cylindrical multi-electrode reactor, specifically designed to generate room pressure non thermal plasma discharges. The reactor is coupled to a stainless steel 135×140×95 mm box post-discharge chamber were the samples to be treated are placed. An ENI ACG-3B RF power supply, operating at 13.56 MHz, sustains the discharge through a PI matching network. A PC data acquisition system is associated to a 460L Teledyne Instruments monitor intended to measure the O₃ concentration in the discharge, while a type-K thermocouple provides a temperature profile with respect to the distance to the reactor outlet.

The cylindrical reactor is structured as an alternate succession of coaxial Pyrex glass tubes and copper mesh covers acting as electrodes, as seen in figure 1, resulting in a multiple capacitive system. Then, the injected 2 SLPM He and 0.066 SLPM O₂ gas mixtures follow a longitudinal flow between every pair of glass tubes with a return trajectory along every second pair of tubes, so as to play the role of a serpentine path. The tube lengths are 60 mm, 65 mm and 70 mm, their external diameters being 14 mm, 22 mm and 30 mm respectively. The effective distance between mesh electrodes is ~4 mm.

The material to be sterilized is placed at 50 mm from the reactor outlet, within the post-discharge chamber, in order to be better exposed to plasma reactive species. In order to validate the capability of the generated plasma to inhibit bacterial growth, an *E. coli* ATCC8739 bacterial strain was inoculated in 5 mL of Luria-Bertani (LB) medium and then incubated for 24 hours at 37°C. Later serial dilutions were carried out on a decimal basis until a $10^4$ CFU/mL concentration was achieved. Finally, sterile Petri dishes containing solid LB agar were inoculated with 0.1 mL of the solution and immediately placed in the post-discharge chamber to be exposed to the plasma species for 2, 4, 6, 8 and 10 minute periods. The treated dishes were incubated at 37°C during 24 hours before counting up the surviving bacterial colonies. Naturally, one untreated dish was included in every set for control purposes.

**Figure 1.** Experimental setup.
3. Results and discussion

3.1. Ozone generation
In order to maximize the O$_3$ volume produced within the reactor with a view to eliminating microbes from surfaces and instruments, ozone concentration was measured at different gas flows. The optimal combination, seen in figure 2, was identified to be 0.066 SLPM of O$_2$ added to 2 SLPM of He, namely, 3.3% of the helium flow, which comes closer to the 97% He + 3% O$_2$ results published in [6].

3.2 Thermal temperature at the reactor outlet
A thermal temperature profile, shown in figure 3, has been constructed by measuring this variable outside the reactor at several distances from its outlet. To this purpose, a K-type thermocouple revealed a range going from 294 K at 10 mm to 322 K at 1 mm, proving that the proposed plasma facility is exempt from producing any thermal damage to the treated objects.

![Figure 2. O$_3$ concentration generated by (A) He (2 SLPM), and its mixtures with: (B) O$_2$ (0.016 SLPM), (C) O$_2$ (0.033 SLPM), (D) O$_2$ (0.05 SLPM), (E) O$_2$ (0.066 SLPM), (F) O$_2$ (0.083 SLPM).](image1)

![Figure 3. Thermal temperature profile with respect to the distance to the reactor outlet.](image2)

3.3. Inhibition of E. coli colony growth
Figure 4 exhibits the effects on the treated samples of the plasma discharge at several power levels and exposure times, as specified on table 1. Clearly, the number of surviving CFU diminishes as the exposure time increases.

4. Conclusions
The capacity of a room pressure glow plasma discharge produced in a multi-electrode DBD plasma source coupled to a post-discharge chamber to inhibit the growth of E. coli bacteria in the order of $10^3$ CFU/ml has been proven. Thus, the sterilization of surfaces without a direct contact with the plasma, and therefore at no risk of material structural damage, is feasible. The proposed plasma facility shows that thermal temperature is not necessarily involved in the elimination of the bacteria provided that samples can be successfully treated at 50 mm from the reactor outlet where the exposure temperature is ~294K, practically the same as the room temperature. Given the high oxidative power of ozone resulting in immediate cell damage, the O$_3$ concentration produced in the discharge has been maximized with purposes of biological decontamination.
**Table 1.** Results from inhibition of *E. coli* population growth.

| Exposure time (min) | 25W  | 30W  |
|--------------------|------|------|
| 0                  | 1635 | 1537 |
| 2                  | 519  | 667  |
| 4                  | 434  | 41   |
| 6                  | 111  | 29   |
| 8                  | 92   | 7    |
| 10                 | 20   | 0    |

Figure 4. Deactivation of *E. coli* in agar by a plasma discharge at different power levels and exposure times.

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