Interaction between an argon-based non-thermal atmospheric pressure plasma jet and Escherichia coli

M. T. Benabbas[1], S. Sahli[1], S. Mouissat[1], N. Kacem Chaouche[2], L. Benterrouche[1], M. Kara Ali[2]

[1]*Laboratory of Microsystems and Instrumentation (LMI), Electronics department, Faculty of Technology Sciences, Frères Mentouri University, Constantine - Algeria*

[2]*Laboratory of Mycology, Biotechnology and Microbial Activity (LaMyBAM), Microbiology department, Nature and Life Sciences Faculty, Frères Mentouri University, Constantine - Algeria*

In this work, some investigations have been carried out on the interaction between an argon-based non-thermal atmospheric pressure plasma jet (ANAPPJ) and Escherichia coli (E. coli) bacteria. The argon gas was injected through the inner electrode of a homemade dielectric barrier discharge reactor linked to a high voltage pulse generator. A plasma jet of length varying from 0 to 6 cm has been obtained by applying a voltage in the range 3 to 5 kV with a signal frequency in the range 10 to 50 kHz. This ANAPPJ has been used to inactivate E. coli bacteria in order to determinate its action field around the impact point on the contaminated surface.

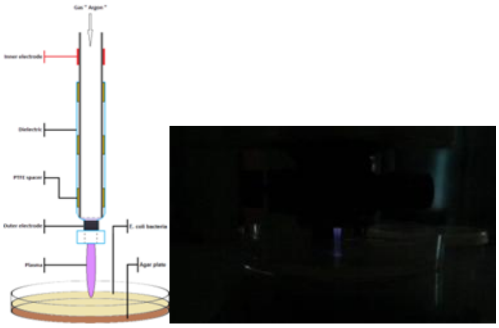
1. Introduction

During the last decade, non-thermal plasmas have been widely studied for several medical applications such as sterilization [1], blood coagulation, teeth whitening [2], dermatology [3] and cancer healing [4]. Atmospheric plasmas created in air or in a noble gas remain the most promising plasmas for biomedical applications because of their safety to use under ambient conditions. The creation of plasma in open air leads to the production, besides the ultraviolet (UV) radiations, many reactive species such as nitrogen oxide (NO), atomic oxygen (O) and ozone (O_3). Several works have shown that these plasma species have high bactericidal effects. Recently, a large number of plasma sources were designed for specific applications in the biomedical field. Among the studied cold plasma sources for medical applications, plasma jet sources are the most attractive. In these kind of atmospheric cold plasmas considered as indirect plasmas, the electrical discharge is created between two electrodes inside a tube and then the used plasma for the treatment is transported in a gas flow until it reaches the area to be treated. The size of the jet can be modulated and can have a small section (less than a millimeter), allowing then the plasma jet to be used for a very located treatment. Although several works have been recently devoted to the plasma jet sources and their potential applications in the biomedical field, however their precision in the treated area remains not well controlled.

It is well known that the plasma jet shape and length are depend on the applied voltage value and its frequency [5], on the surrounding air humidity and the nature of the working gas [6 - 7]. In this work, we have generated at room temperature and humidity, an argon-based non-thermal atmospheric pressure plasma jet of about 27 mm of length. We have exposed an E. coli contaminated surfaces to this plasma jet. In the following, we will present the effect of the plasma source – sample distance variation and the effect of exposure duration to this plasma jet, on the diameter of the inhibition zone created on the E. coli contaminated surfaces.

2. Experimental details

The schematic representation of the homemade argon-based non-thermal atmospheric pressure plasmas jet reactor is reported in Figure. 1. It consists of a stainless steel tube of 6 mm and 1 mm of external diameter and thickness respectively, (the inner electrode) inserted into a glass tube of 1 mm of thickness (the dielectric barrier) and a coiled tungsten wire of 0.5 mm of diameter placed around the neck of the glass tube (the outer electrode). The argon gas was injected through the inner electrode to the discharge area situated between the two electrodes. This geometry prevents glow to arc transition due to charge propagation. The inner and the outer electrodes are linked to a high voltage pulse generator Redline G2000 (Redline Technologies Elektronik GmbH, Baesweiler, Germany). The plasma jet monitoring was assured by Ocean Optics HR2000+ES (Ocean Optics, Inc., Florida, USA). The studied samples were constituted from substrates of agar plates contained in petri dishes and contaminated by E. coli bacteria spread out on their surface and then dried at room temperature. The samples were exposed to the plasma jet during different times and at different distances from the source of the plasma jet. A Canon PowerShot SX220 HS camera (Canon Inc., Tokyo, Japan) was used to take photos of the treated samples. During all the experiments, the applied peak voltage and its frequency were fixed at 4 kV and 18 kHz respectively.



(a) **(b)**

Figure. 1: Schema (a) and photo (b) of the homemade plasma jet set up.

3. Results and discussion

3.1. Effect of the distance variation between the plasma jet nozzle and the contaminated surface

The contaminated samples placed at 10, 20, 30 and 40 mm away from the nozzle of the plasma jet (27 mm of length) were exposed for 10 min to the plasma jet. At a distance of 10 and 20 mm, the samples were completely immersed in the plasma jet and by consequence, directly exposed to the jet (direct mode). At 30 and 40 mm, the contaminated surface was located far from the plasma jet extremity of about 3 mm and 13 mm respectively (indirect mode). Figure. 2 shows the inhibition zone and Figure. 3 shows the evolution of this last versus the distance between the contaminated surface and the plasma jet nozzle. The increase of this distance induces a decrease of the I.Z.D created on the treated samples. From a value of about 7 mm obtained at a distance of 10 mm from the plasma jet nozzle, the I.Z.D decreases to near 0 mm for a sample treated at 40 mm far from the nozzle. The presence of this deserted area observed for distances lower than 40 mm from the plasma nozzle, shows a disappearance of the bacteria from the treated zone because of cadaver consumption by the plasma species created in the argon gas and the ambient air. Many works have revealed that the interaction of bacteria with a cold plasma may have different effects leading to their inactivation and/or removal from the contaminated surface. UV radiations emitted by the plasma can damage the DNA of the bacteria cells causing their inactivation, the reactive species react chemically with their membranes inducing an oxidation of their constituents and the electrostatic forces produced by the charged particles of the plasma accumulated on the outer surface of the bacteria cells, can induce the rupture of their membrane [8 - 10].

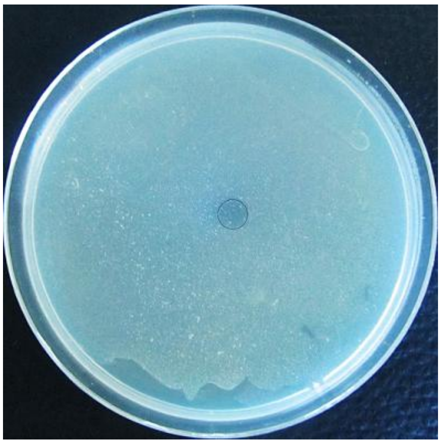


Figure. 2: Photo shows the inhibition zone on an E. coli contaminated surface.

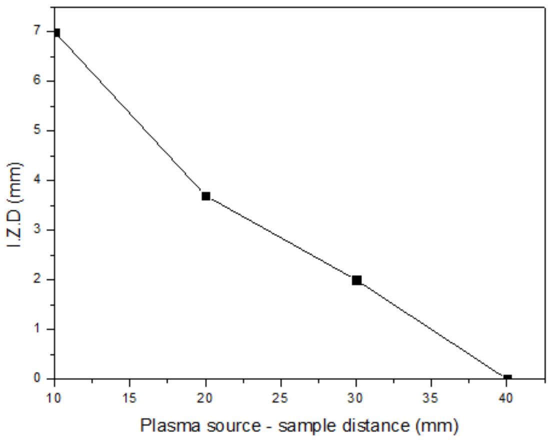


Figure. 3: Effect of the distance between the plasma jet nozzle and the contaminated petri dishes surface on the inhibition zone diameter (exposure time: 10 min).

The variation of the I.Z.D depends on the plasma jet diameter and species nature, energy and density. In the direct mode (at 10 and 20 mm, the effect of these parameters is more pronounced when the treated surface is close to the nozzle and decreases when the contaminated surface is placed far from the nozzle. In the indirect mode (samples placed at 30 and 40 mm), since there is no contact between the plasma jet and the treated samples surface, charged particles have little effect on the bacteria [11]. The removal bacteria from only a little area on the contaminated surface may be due to the presence of less reactive oxygen species reaching the petri dishes surface compared to that produced in the direct mode. Especially, the ozone created by the plasma can have an important effect because of its longer lifetime compared to that of atomic oxygen O and OH groups [12].

3.2. Effect of the exposure time

Petri dishes containing E. coli bacteria spread out on agar plates were immersed in a plasma jet at 10 mm from the plasma jet nozzle, during different treatment times. The plasma jet creates a circular zone without any visible E. coli microorganisms. The diameter of this zone increases with the increase of the exposure time to the plasma jet, showing then more bacteria inactivation. On Figure. 4 is represented the variation of the diameter of the inhibition zone induced by the plasma jet as function of exposure time.

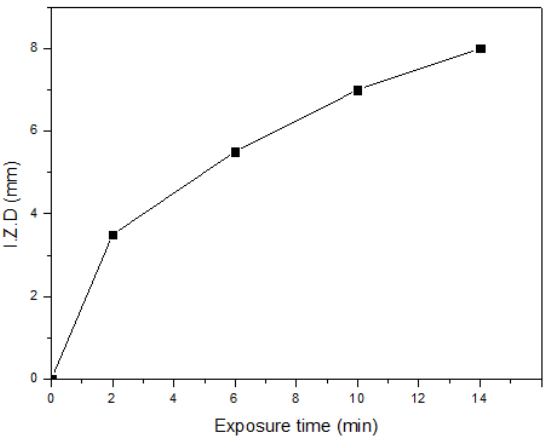


Figure. 4: Effect of the exposure time on the inhibition zone diameter (distance from the plasma jet nozzle: 10 mm).

When the exposure time increases from 0 to 14 min, the I.Z.D curve variation shows two behaviors. The first one in the treatment time range 0 – 6 min, presents a pronounced I.Z.D increase (from 0 to near 5.5 mm) and the second one in the range 6 – 14 min, shows a less pronounced I.Z.D increase (from 5.5 to about 8 mm). Beyond 14 min, a quasi-saturation of the I.Z.D is reached. This quasi-saturation of the I.Z.D can be explained by the short lifetime of the plasma species at atmospheric pressure. Because of the high pressure, the plasma species cannot diffuse sufficiently far from the center of the impact zone and lose their reactivity, even if the treatment time is increased.

4. Conclusion

An argon-based non-thermal atmospheric pressure plasma jet (ANAPPJ) has been generated using a homemade DBD-based plasma jet reactor and a high voltage pulse generator. This ANAPPJ has been used to inactivate E. coli bacteria in order to determine its field of action. The efficiency of the plasma jet and in E. coli inactivation process and its field of action depend on exposure time, treatment mode (direct or indirect) and plasma source – sample distance. A plasma jet with a controlled action field diameter can be obtained and allows then the use of this plasma jet for a very localized treatment.

5. Acknowledgments

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6. References

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