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Comparative study of bacteria sterilization by cold plasma produced by DC generator with positive and negative polarity using atmospheric pressure

***Abstract**. In this paper several studies were carried out using a sterilization method with a corona electrical discharge plasma. A tip-to-plane configuration were used with negative and positive polarity power supplies to perform a parametric sterilization studies on E. coli (ATCC 25922) and Bacillus subtilis (ATCC168) bacteria. Plasma has several effects, but the most important are the bacteria destruction on packaging surface and food products for safer products and less likely to be contaminated during longer storage, and the non-aggressive sterilization of heat-sensitive surgical instruments such as endoscopes. These studies present the bacteria resistance and interaction phenomenon by each polarity of the direct current (DC) plasma source on the surfaces. The obtained results show that relatively short treatment time of the order of few minutes is sufficient to remove a significant wet charge of E. coli (Gram G-) by positive polarity and only 11 min by negative polarity. On the other hand, only 65% of Bacillus subtilis (Gram G+) charge was sterilized by positive polarity and 12% by negative polarity for 40 min treatment time. To conclude this work, a comparison was made between the two polarities.*

***Streszczenie.** W artykule przedstawiono szereg metod sterylizacji z wykorzystaniem plazmy przy wyładowaniu koronowym. Badano efekt przy różnych rodzajach bakterii. Stwierdzono pełną sterylizację po czasie od kilku do kilkunastu minut. Ale w przypadku niektórych bakterii nawet 40 minut dawało efekt tylko w 65%. (**Analiza porównawcza metod sterylizacji z wykorzystaniem plazmy powstającej przy wyładowaniu koronowym DC**)*

Keywords: Sterilization – bacteria, corona discharge , cold plasma, atmospheric air
Słowa kluczowe: sterylizacja, plazma, wyładowanie koronowe

Introduction

In recent years, the electrical discharge plasma applications at atmospheric pressure are extensively emerging in biology, agriculture and medicine [1,2]. In fact, the atmospheric pressure plasma use is more appropriate and easy to perform then complex low pressure plasma technologies [3]. The activity in this direction form a plasma chemistry new field called "Plasma medicine". Some medical applications examples of plasma are dental cavities treatment [4,5], different surfaces sterilization [6, 7], skin diseases treatment [1-6], [8-9], and delicate surgeries [5-10], [11]. Now, it is clear that these plasmas can not only have physical effects on surfaces but can also have complex effects on biological field such as living cells and microorganisms [12]. The purpose of this article is to perform a parametric study to propose a first model of a tip-plane apparatus to be used for the sterilization of medical instruments.

Fridman & al stated that the direct plasma which contact with the tissue results significantly a faster bacteria inactivation on this later due to charge presence [13]. If the corona discharge occurs from a negative polarity in air at atmospheric pressure and relative humidity between 40% and 60 %,in the first hand ,the negative ions will form CO_3^- et O_2^- and in the other hand, the positive ions will forme $(H_2O)_n H^+$, NO^+ , NO_3^+ when the corona electrodes polarity is positive [14,15]. Some plasma devices are designed to operate specifically by creating large flows of NO [16]. The role of NO generation of medical plasma devices has recently been examined [17,18]. There has been a considerable effort in the development of various therapeutic NO^- donors either through compounds that break down to form NO or through some other mechanism [19,20]. In the plasma medicine context, the well-known role of NO in wound healing and its antimicrobial role is generally stressed. But there is also growing interest in NO and related compounds for its anticancer applications [21]. Recently, it has been shown that not only the reactive generated species in the plasma are responsible for achieving the effect of sterilization but also the charged species (electrons and ions). To this end, bacteria sterilization study by two DC power sources with negative and positive polarity was the subject of this modest work, as well as their evaluation of their effect on bacterial inactivation and other biological effects.

Materials and methods

Installation and procedure

Fig.1 shows the schematic diagram of the facility used for the study of plasma sterilization. The system is composed of A needle tip configuration for bacteria treatment by plasma (1), two high voltage (HV) DC generators (40 kV, 38 mA) with different polarity negative and positive (2), an autotransformer for varying the HV output (3), an 8 MΩ resistor to limit the discharge current (4), a divider probe to measure the output voltage (5), a 7kV electrostatic voltmeter used with the probe measurement (6), a micro-ammeter to measure the current (7) and two Bunsen burners to provide a sterile work area(8).

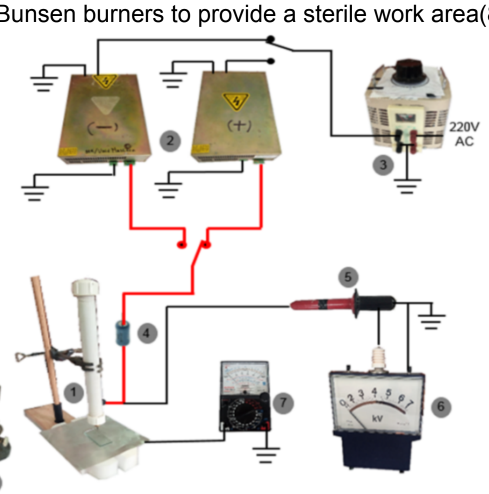

Fig. 1. Experimental setup. (1) Tip-plane configuration, (2) two high-voltage DC power supplies with negative and positive polarity, (3) Autotransformer, (4) Resistor of 8 MΩ, (5) Probe, (6) Electrostatic voltmeter, (7) Micro- ammeter, (8) Bunsen burner.

Fig. 2 shows the current-voltage characteristic of the DC power supply with negative and positive polarity used for sterilization in the LSTE laboratory under atmospheric conditions (temperature 20 °C and relative humidity RH% between 30 and 50%).

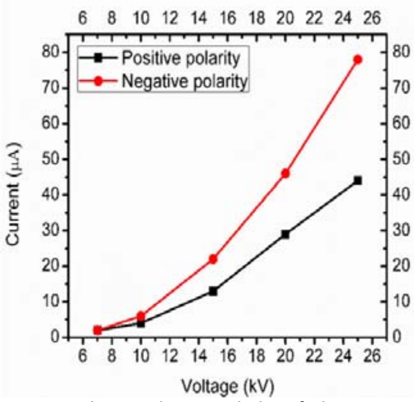

Fig. 2. Current - voltage characteristic of the power supply for negative and positive polarity

The bacteria E. Coli G- (ATCC 25922) and Bacillus Subtilis G + (ATCC 168) were chosen in order to study the bacterial inactivation. In each experiment, one drop of this bacterial load is measured with a Shimadzu Europe -UV-mini 1240 spectrometer shown in Fig. 3 and then placed on a sterile glass for plasma treatment. After treatment, several dilutions of the bacterial load treated with plasma are made (up to 10^{-6}), then from each dilution, samples are taken in Petri dishes and placed in an incubator at 37 °C for 24 hours. (see Fig. 3)

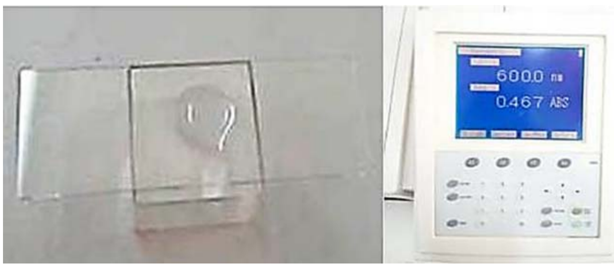

Fig. 3. Photography of E. coli liquid drop with optical density in the spectrometer UV-mini 1240.

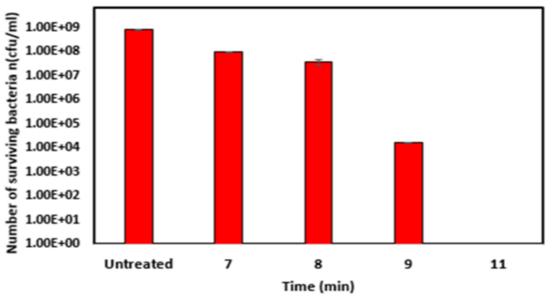

Fig. 4. Evolution of the number of bacteria as a function of plasma treatment time. Corona Voltage = 25 kV, Interelectrodes distance = 30 mm, Negative polarity

Results and discussions

Parametric study at Negative polarity

Treatment time effect

In this study, the E. coli bacteria were exposed to different treatment times of 7, 8, 9 and 11 min, the other parameters are fixed such as 25 kV voltage and a 30 mm distance. Fig. 4 shows that increasing treatment time will reduce the bacteria number. Interestingly, it seems that after 11 min of treatment, more than 8.10 * 10^8 (cfu / ml) of bacteria are sterilized (see Fig. 5). The decrease in the number of surviving bacteria as a function of treatment time is not linear. The same effect was observed in other studies [12]. This observation is very interesting because it show the complexity of the plasma sterilization effect on bacteria and need to be more investigated from the microbiology point of view.

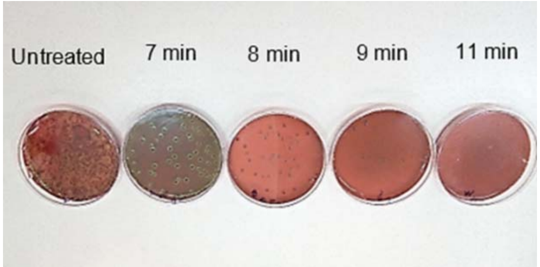

Fig. 5. Photography of bacterial load as a function of plasma treatment time

Plasma temperature effect on bacteria load

To see the thermal effect on the wet load during sterilization, a probe is used to measure the temperature versus treatment time (see Fig. 6 and 7).

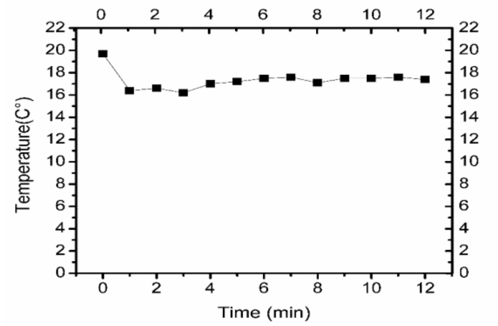

Fig.6. Characteristic of temperature effect on the load Corona Voltage = 25 kV, Interelectrode distance = 30 mm.

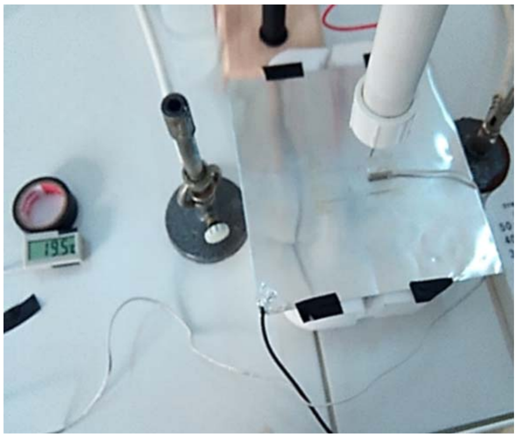

Fig. 7. Photography of the installation used for the temperature effect measurement on the load

Fig. 6 shows that treatment time does not affect the temperature in this case. In fact, it seems that the plasma treatment is happening at ambient and constant temperature. This phenomena is due to the ionic wind which is generated at the high voltage tip electrode. The ionic wind is flowing from the tip electrode toward the bacteria load and act as a fan or cooler. From Fig. 6 it can be seen that that bacteria load temperature at the beginning of treatment was at 20°C and it dropped after 1 min to 16°C due to ionic wind cooling effect. The temperature has increased after that under the effect of plasma treatment but it does exceed the18°C. The plasma treatment is performed at room temperature.

Corona Voltage effect

In this test, E. coli bacteria were exposed to corona discharge plasma at three different voltage levels 13, 17 and 21 kV. The other parameters were fixed, such as 11 min treatment time and 30 mm distance between needle tip and the sample. Figures 8 and 9 shows that the increase in the supply voltage has a positive effect on the sterilization of the sample. By comparison between the results of Fig. 8 and Fig. 4 it can be observed that for higher Corona voltage than 21 kV the bacteria load will be completely sterilized. This result shows that the voltage effect is also non-linear. This fact must be investigated on a microbiologic scale to determine the reason behind this type of complete sterilisation. The complexity of the plasma – bacteria interaction phenomena is to be more investigated. Also a chromatographic analysis of plasma gases in correlation with the present results will be the subject of future paper.

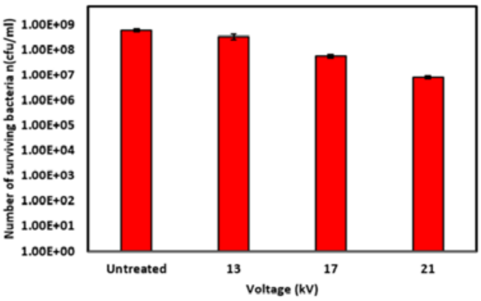

Fig. 8, Discharge voltage effect on the bacteria number. Treatment time = 11 min, Interelectrodes distance = 30 mm, Negative polarity.

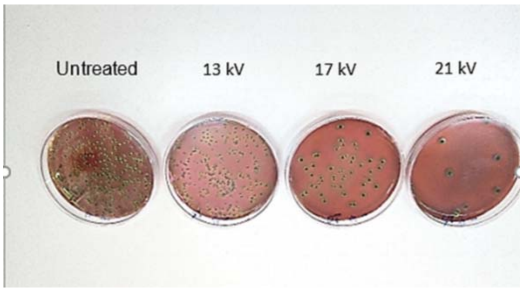

Fig. 9. Photography of bacterial load versus voltage.

The tip/bacteria sample distance effect

E. coli bacteria was exposed to plasma for different distances between needle tip and the samples (25, 30, 35 and 40 mm). The other parameters are fixed, such as 25 kV voltage and 11 min time treatment. Fig. 10 shows that increasing the distance also has a negative effect on the of bacteria number. In Fig. 11, we note that 25 and 30 mm distance with 11 min treatment time complete the total sterilization by approximately 10^9 (cfu / ml).

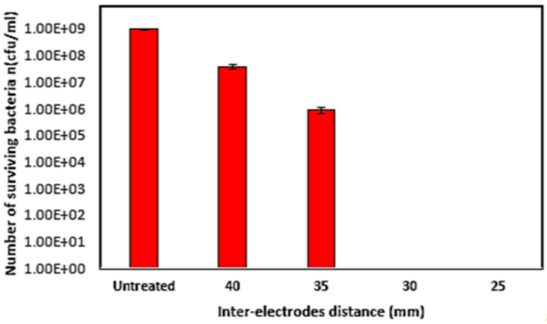

Fig. 10. Bacteria number versus tip/bacteria sample distance. Treatment time = 11 min, Corona Voltage = 25 kV, Negative polarity.

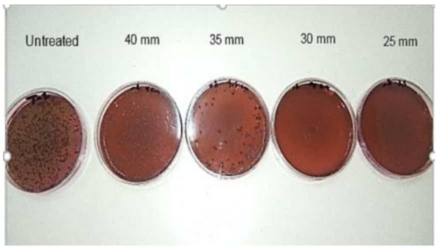

Fig. 11. Photography of bacterial load versus tip / bacteria sample distance

Fig. 12 shows that the discharge current decreases with increasing distance. This mean that for high distances the plasma is weak and the reagent species quantity is low which lead to a decrease in the sterilization efficiency.

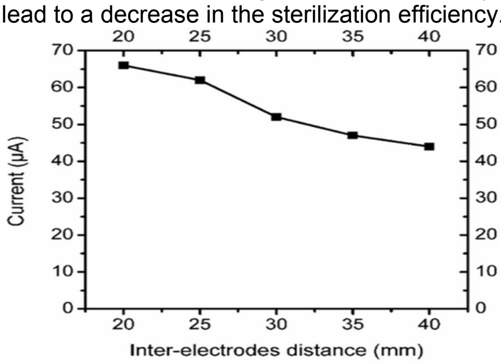

Fig. 12. Electrical discharge current evolution versus the interelectrodes distance. Corona Voltage = 25 kV, Negative polarity.

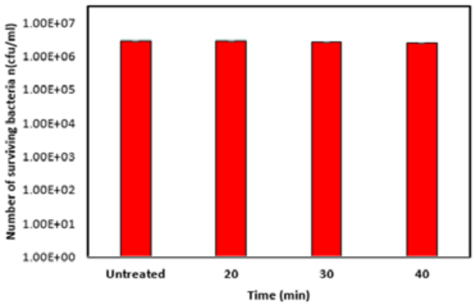

Fig. 13 Bacteria number evolution versus plasma treatment time. Corona Voltage = 25 kV, Interelectrodes distance = 30 mm, Negative polarity.

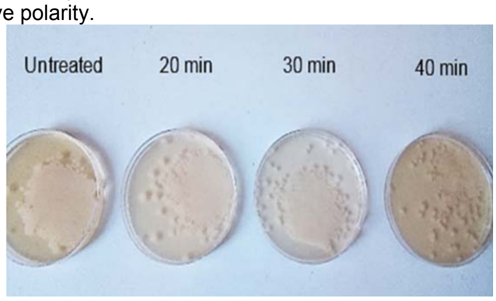

Fig. 14. Photography of bacterial load versus plasma treatment time

Treatment time effect on Bacillus subtilis bacteria

In this study, Bacillus subtilis bacteria were exposed for 20, 30 and 40 minutes, the other parameters are fixed such 25 kV voltage and 30 mm distance. Fig. 13 and Fig. 14 shows that the increase in treatment time slightly reduces the bacteria number. Except 12.19 % of the control bacteria number were sterilized after 40 min of treatment.

Parametric study at Positive polarity

Treatment time effect

In this experiment , E. coli bacteria were exposed for 1, 2 and 3 min of treatment time. The other parameters are fixed such that 25 kV voltage and 30 mm distance. Fig. 15 shows that the increase in time reduces the bacteria number. Fig 16 shows also that after 3 min of treatment time, more than 8.20 * 10^8 (cfu/ml) bacteria are sterilized.

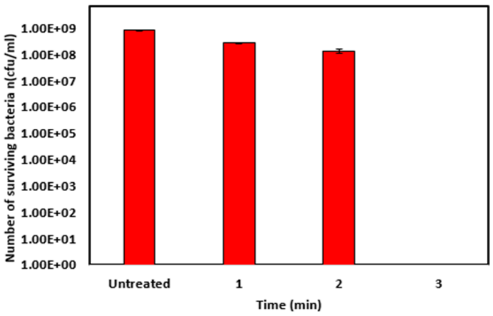

Fig. 15. Bacteria number evolution as a function of plasma treatment time. Corona Voltage = 25 kV, Interelectrodes distance = 30 mm, Positive polarity.

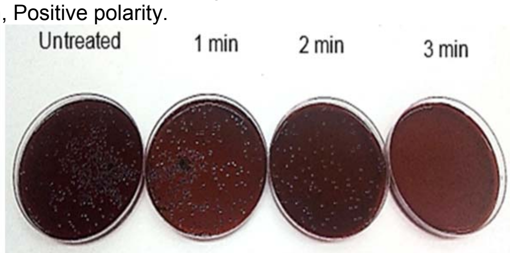

Fig. 16. Photography of bacterial load versus plasma treatment time

Treatment time effect on Bacillus subtilis bacteria

The bacteria Bacillus Subtilis were exposed for 20, 30 and 40 min of treatment time. The other parameters are fixed such 25 kV voltage and 30 mm distance. Fig. 17 shows that the increase in treatment time reduces a significant number of bacteria. Fig. 18 shows that after 40 min treatment time, 65. 32% of control bacteria were sterilized. This is better than the negative polarity results. This observation confirm that the plasma generated in the negative polarity is not the same as that generated in positive polarity. The gas chromatography will be performed in the next work to determine the nature of the species that are boosting the sterilization effect.

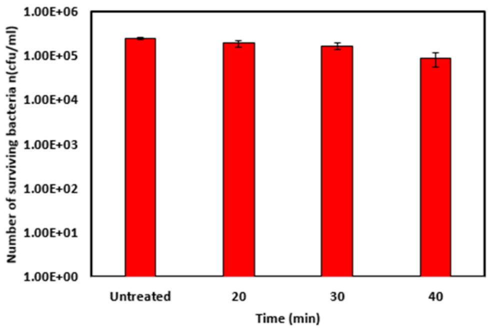

Fig. 17. Bacteria number Evolution versus plasma treatment time. Corona Voltage = 25 kV, Interelectrodes distance = 30 mm, Positive polarity.

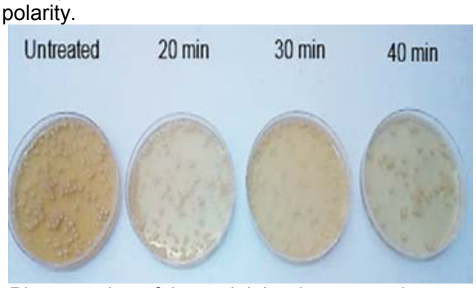

Fig. 18. Photography of bacterial load versus plasma treatment time

Comparison between positive and negative polarity

In this comparison a bacteria liquid load was treated for 6 min at 25 kV of corona DC voltage and with a distance of 30 mm between the tip of the needle and the bacteria load. Both polarities were used under the same atmospheric conditions. Fig. 19 shows that the bacteria is completely sterilized for the positive polarity at 6 min while it requires more time to obtain the same effect for the negative polarity. The positive polarity treatment in time is more efficient than in the negative polarity.

Fig. 19. Photography comparing negative and positive polarity treatment effect. Corona Voltage = 25 kV, Interelectrodes distance = 30 mm

Sterilized surface diameter

To determine the sterilization area of each needle point, E. coli is spread in the agar and cold plasma sterilization is applied directly in the petri dish (see Fig. 20).

The sterilized surface is clear and uniform and reaches a diameter of 20 mm in less than 5 min of treatment time (see Fig. 21). This measurement can help to design an optimized sterilization instrument.

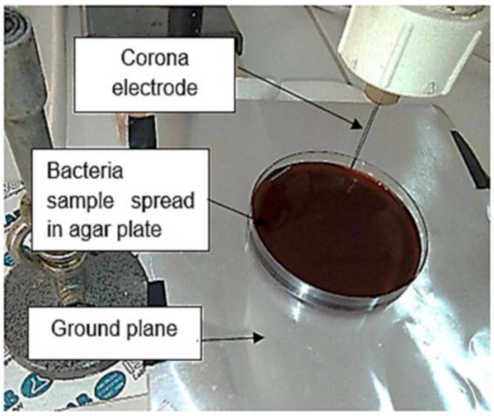

Fig. 20. Photography of E. Coli Sterilization on the agar surface.

Fig. 21. Photography of the sterilization field on the surface after 5 min. Negative polarity.

Conclusion

In this paper, bacteria inactivation using a plasma jet at atmospheric pressure produced by a DC generator with two polarity with a needle / plane configuration was studied experimentally. It was found that sterilization effect and electrical discharge interaction of point to needle / plane configuration with E. coli bacteria is not linear. The corona electrical discharge interaction with atmospheric air and the generated gas species forming the ionic wind flow using a DC source with positive polarity can considerably improve sterilization efficiency. A 3 min treatment time was sufficient for the positive polarity because of the different nature of generated gases species. However, corona electrical discharge sterilization of negative polarity is uniform and the much more clear and reproducible compared to positive polarity. Thus, this technology has promising prospects for sterilization in food and surgical-medical sectors. For future work, a chromatographic gas analysis will be performed on the ionic wind in correlation with the results of the present paper. Considering the current international pandemic situation, the goal is to design an optimized corona sterilization apparatus that can be easily used anywhere there is a necessity of sterilization.

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