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Inactivation of Air-Suspended-Cells Through Ion Energy

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Abstract: Cold plasma at room temperature and pressure is an emerging and novel tool for the inactivation of cell derived from its ionic energy, which is capable of breaking bonds such as carbon-carbon, carbon-hydrogen, carbon-oxygen, among others, these being the ones that support the biological structure of microorganisms. This work tries to create its own infrastructure that includes a high voltage source, an infrared trigger which reduces the effect of the ozone created, an ionic reactor in which the plasma acts at atmospheric temperature and pressure, and a magnetic field that allow aligning the plasma to the combined processes that are carried out.

Key words: Cold plasma at room temperature and pressure, inactivation of cells, ionic energy.

1. Introduction

Air contamination has always been a motive to seek innovation and use new technologies to assure good air quality. Through the past years, bacteria resistance has been a problem and still is an issue of importance, the health perspective of this concept tells us that inactivation of bacteria might be done by using other methods rather than UV (ultraviolet) and drugs with bactericidal performance. Cold plasma is an emerging, efficient and promising technology that also works as a bactericidal alternative [1-3].

2. Materials and Methods

It was managed to design a reactor that was able to work with a high energy ionization, whose source can generate a voltage between 25-30 kV, creating cold plasma at room temperature and pressure. The cold plasma discharges are channeled towards three meshes that act as electrodes in which microorganisms, such as *Escherichia colli* and *Pseudomona aeruginosa*, are immersed [3-4].

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During the experiments, the voltage, frequency and time were able to be varied, thus, their affectations on the microorganism were analyzed biologically [1, 3, 5].

2.1 Bacterial Culture and Media

The strains of *E. coli* ATCC 25922, and *Pseudomona aeruginosa* were provided from Thermo Scientific. The bacterial cultures were maintained frozen at -20 °C in refrigeration.

The medium culture was prepared using dehydrated agar for standard method (the medium culture was bought online and provided by DIBICO S.A. de C.V.). Considering that the amount used was for making only 0.5 L of agar, 11.5 g of agar was mixed with deionized water while heating until it reached boiling point and let it boil for 1 min. After that it was sterilized for 15 min at 121 °C [4, 6].

The nutrient solution was prepared according to the procedure mentioned in MGA 0571 pharmacopoeia microbial limits. One point eight grams (1.8 g) of monobasic potassium phosphate, 3.6 g of dibasic

sodium phosphate, 2.15 g of sodium chloride, 0.6 g of peptone casein (the reagents were bought online and provided by DIBICO S.A. de C.V.) and 0.5 L of deionized water were mixed and then sterilized at 121 °C for 15 min [7].

The pH at the beginning and after the sterilization must be inside the range of 7.0 ± 0.2 at 25 ± 2 °C.

Petri dishes were used to hold the agar where it was going to work as a solid culture medium. *E. coli* and *Pseudomona aeruginosa* strains were inoculated into 11 mL of the culture medium using the crossed striae technique and incubated for 24 h at 32 °C, which resulted in stationary-phase cultures [6].

2.2 Analytical Measurements

Both, *E. coli* and *Pseudomona aeruginosa* suspensions were prepared by inoculating some (μL) of the stationary-phase culture into 9 mL of fresh nutrient solution [7].

The *E. coli* sample was measured and evaluated using quartz cuvettes (using a SPECTRO UV-VIS dual beam of 8 auto cells from labomed, inc.) until the transmittance reached 80%. The transmittance coefficient of the sample was determined from the intersection of the transmittance vs. wavelength (nm), at 580 nm [7].

For the *Pseudomona aeruginosa* sample, the transmittance was also measured, obtaining a coefficient value of 0.1%. Once the concentration was known on both samples, the procedures to make the dilutions are explained next:

(1) 1 mL of the *E. coli* solution that was measured at 80% in transmittance was grabbed and was injected on the first tube (1) so it could have a value of 1×10^{-1} of the initial concentration, subsequently after it was mixed, we grabbed 1 mL of the tube 1 and injected on the second tube (2) so it could have a value of 1×10^{-2}. It was done consecutively until the 8th tube (8) that would have a value of 1×10^{-8} (Fig. 1).

(2) The same thing was done for the *Pseudomona aeruginosa* solution.

(3) For both, *E. coli* and *Pseudomona aeruginosa* dilutions, 1 mL from the tubes 2 and 8 was grabbed and was inoculated into 11 mL of medium culture each and let it incubate for a period of less than or equal to three days in a temperature range of 30-35 °C.

(4) The rest of the remaining dilutions were centrifuged so it was able to separate the bacteria particles from the dilution and those particles were passed inside the plasma reactor at different times and conditions.

2.3 Incubation of Treated Samples and Survival Counting

Samples recovered after crossing the plasma reactor, were incubated for 24 h at 32 °C. Previous experiments demonstrated that cold plasma can inactivate some bacteria's yet not all the bacteria concentration of the samples, so it was important to see how many colonies were able to grow. The samples treated were inoculated

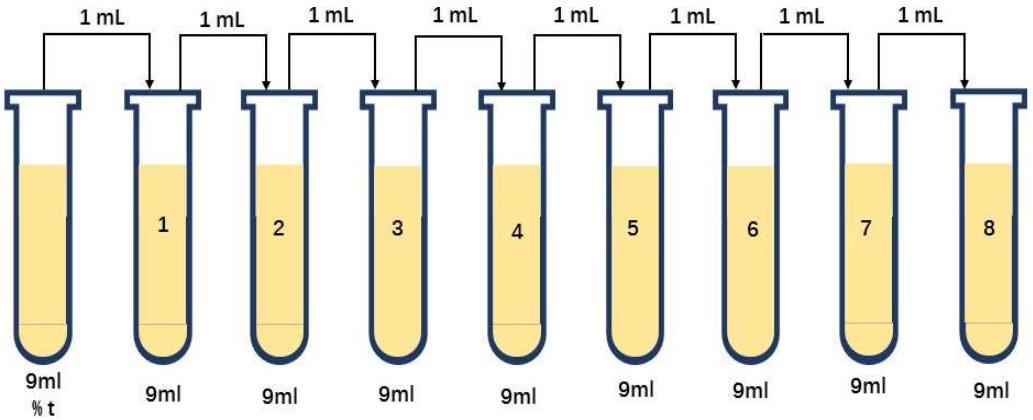

Fig. 1 A scheme of the dilutions that were made so we could have a count of the colonies that would grow.

in a solid culture medium and left in incubation for 24 h. After incubation, CFU (colony forming unit) was counted by using bare eyesight.

2.4 High-Voltage Power Supply

The voltage generated by the source was between 12 kV and 31 kV. The frequency can vary between 5 Hz and 200 kHz. The power supply is 24 V batteries [8, 9].

In Fig. 2, we can see that the plasma system works as a capacitor, where energy has to be stored to be able to ionize the air, this is because the experiment was working with plasma at atmospheric temperature and pressure.

Fig. 3 shows the diagram of the power supply, starting with the generation of the frequency.

Fig. 4 shows corresponds to the sketch of the high power phase.

Fig. 5 shows the diode arrangement allows to keep the high voltage supply stable.

This circuit allows the frequency to be varied from low-frequency values to high-frequency values.

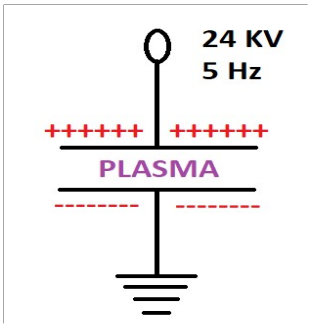

Fig. 2 Plasma functioning as a capacitor.

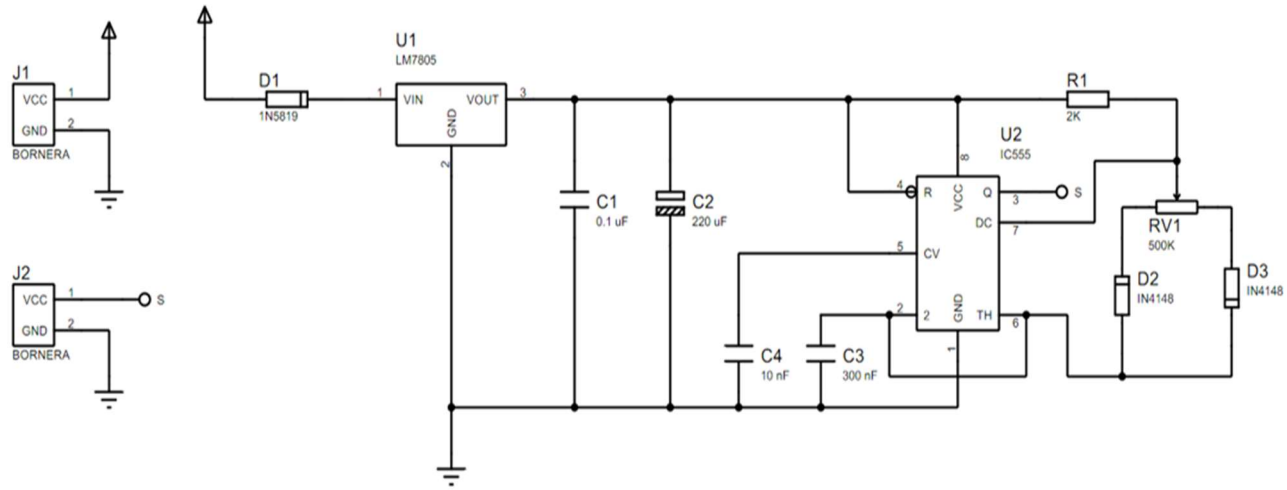

Fig. 3 The power supply.

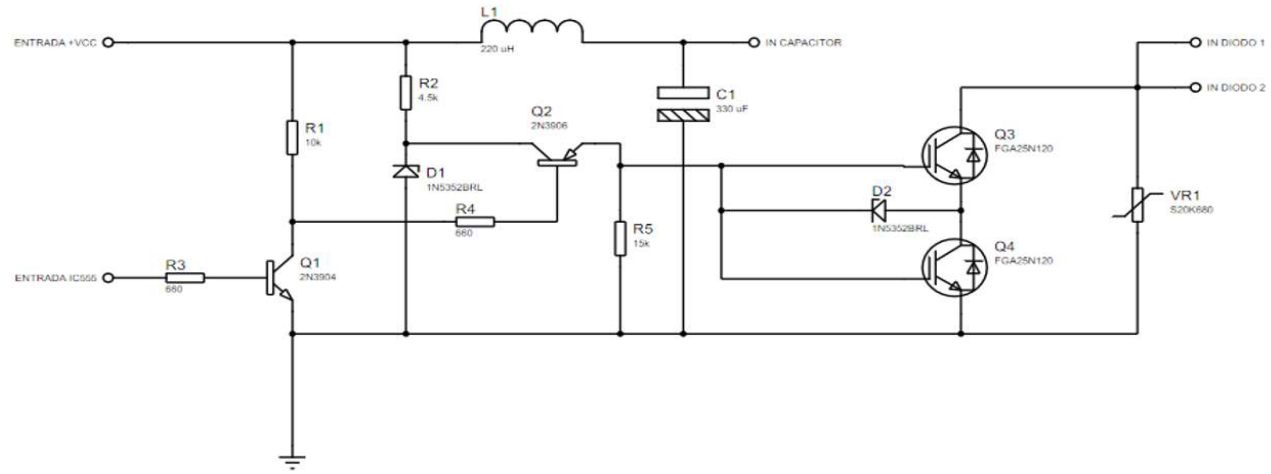

Fig. 4 Voltage source.

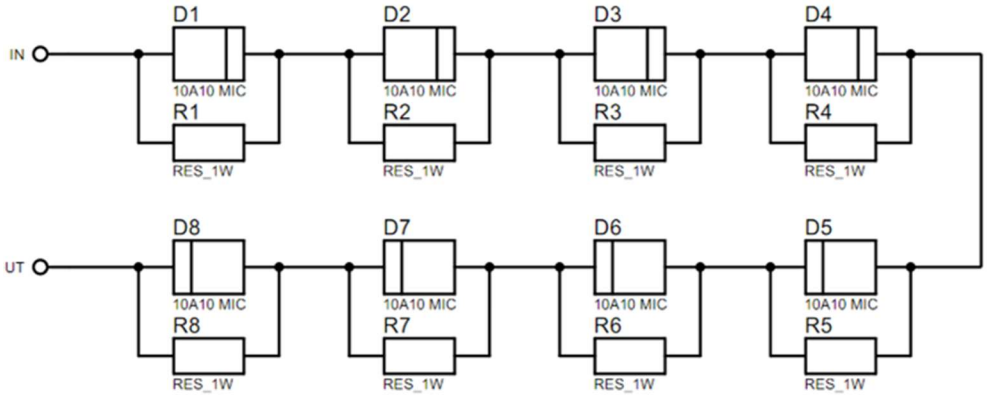

Fig. 5 Diode array.

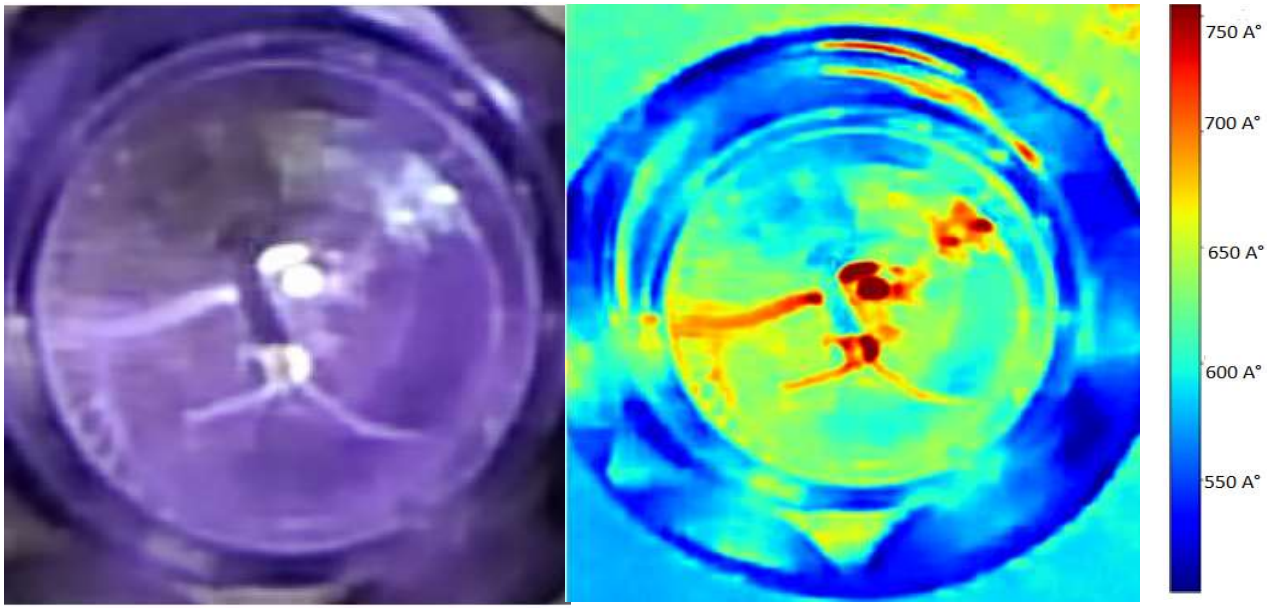

Fig. 6 Effects of the cold plasma inside the reactor.

The output of this power circuit is added to an array of diodes and resistors, where we can dissipate the energy from the high-voltage coil.

2.5 Cold Plasma Characterization

The cold plasma obtained was characterized using HEIF (high-efficiency imaging) using a Galaxy S20+ cell phone, 64 MP, f/2.0, OIS (optical image stabilization), with 30× digital hybrid optical zoom [10, 11].

The treatment of the images was carried out using Python 3.9 at 64 Bit with its Spyder editor and with an NVIDIA graphical interface ver. 3.25.1.27 [12, 13].

To reduce the effects of ozone, an ultraviolet light trigger was built, synchronized by a stepper engine that moves a perforated disc which works as a shutter.

The experiments were carried out in a dark room to avoid interference with light [3].

The ionization reactor is immersed in electromagnetic fields that induce and homogenize the plasma.

3. Experimental Results

Fig. 6 shows in the left image the effects of the cold plasma inside the reactor acting on the microorganisms, on the right the treatment with red, green and blue filters, these filters correspond to wavelengths between 750 A° and 500 A° corresponding to the braking of the bonds C-C, C=C, C=O, C-H, among others.

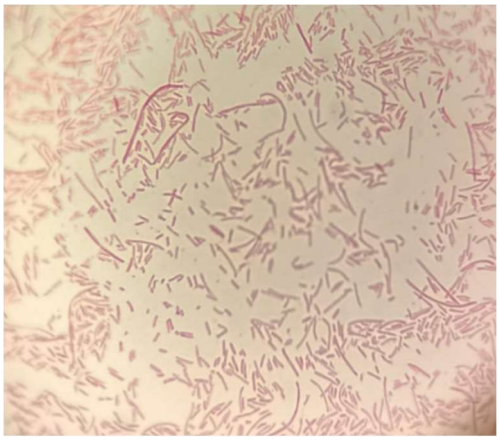

Fig. 7 Sample of *E. coli* in the initial conditions.

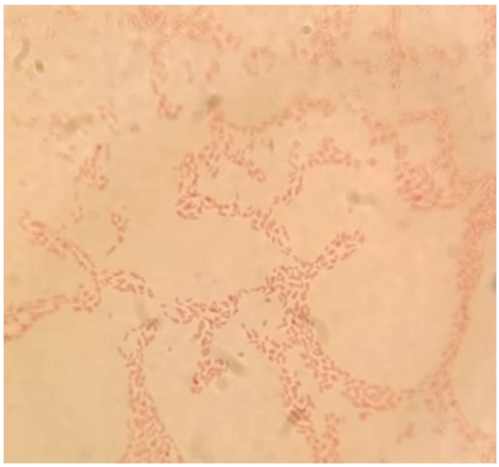

Fig. 8 Sample of *Pseudomona Aeruginosa in the initial condition.*

In Fig. 7, the E. *coli* show medium size, beige color, elongate form and brilliant light reflection, opaque light transmission and consistency creamy.

In Fig. 8, the *Pseudomona Aeruginosa* show medium size, beige color, circular form, bright light reflection, opaque light transmission, creamy consistency and viscous elevation.

A high-voltage power supply was built from a coil that allowed the voltage and frequency to be varied.

An infrared light trigger was built to reduce the effects of ozone.

Different microorganism was incubated to be experimentally tested in the plasma reactor.

The colonies on both *E. coli* and *Pseudomona aeruginosa* that were able to grow in a culture medium were minor than the number grown before entering the reactor. This tells us that cold plasma used as a bactericidal alternative is efficient yet needs to be explored more.

4. Conclusions

The necessary infrastructure was built for the development experimental with cold plasma at atmospheric temperature and pressure.

An infrared trigger was built to reduce the effect of the ozone created.

A plasma at atmospheric temperature and pressure was generated by acting with a magnetic field that allowed the plasma to be aligned to the combined processes that are carried out.

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