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Influence of Distance and Argon Flow rate on Pseudomonas aeruginosa Bacteria Exposed to Non thermal Plasma at Atmospheric Pressure

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Abstract

In this research, a type of gram negative bacteria was exposed to non-thermal plasma at a distance of (2 and 3 cm) from the plasma flow nozzle, with the use of an alternating power supply (5KHz), where exposure was made at two different voltages (4.9 and 8 kV). A negative gram of *Pseudomonas aeruginosa* bacteria was isolated and exposed to non-thermal plasma at different flow rates of argon gas whose value ranged from (1-5) liters/minute. The results showed that bacterial killing rate is directly proportional to distance while exposing the samples to non-thermal plasma, and the best factors by which a complete killing rate was obtained were at a distance of 2 cm with a voltage of 8 kV and a gas flow rate of 5 liters/min, while complete killing of bacteria was not achieved at a distance of 3 cm where the percentage was 95% at the same argon gas flow rate.

Keywords: Atmospheric pressure, High voltage, Non-equilibrium Plasma, Plasma jet, *Pseudomonas aeruginosa* bacteria.

تأثير المسافة ومعدل تدفق غاز الارجون على بكتيريا سيدوموناس ايروجينوسا المُعرضة بالبلازما غير الحرارية عند الضغط الجوي

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الخلاصة

في هذا البحث تم تعريض نوع من انواع البكتريا السالبة للبلازما غير الحرارية عند مسافة (2−3 سم) عن فوهة التدفق للبلازما، مع استخدام مجهز قدرة متناوبة حيث تم التعريض عند قيمتين مختلفتين للفولتية العالية (٤,٩ – 8 كيلو فولت)، تم عزل بكتريا الزائفة الزنجارية سيدوموناس ايروجينوسا السالبة لصبغة غرام وتعريضها للبلازما غير الحرارية عند مستوى تدفق مختلف لغاز الارجون تراوحت قيمته من (١ – 5) لتر/دقيقة، حيث بينت النتائج ان لتأثير المسافة تناسبا طرديا مع نسبة القتل في البكتيريا اثناء تعريض العينة للبلازما غير الحرارية، وان افضل العوامل التي تم الحصول من خلالها على نسبة قتل تام كانت عند المسافة 2 سم مع فولتية 8 كيلو فولت ومستوى تدفق 5 لتر/دقيقة بينما لم يتحقق القتل التام للبكتيريا عند مسافة 3 سم حيث كانت النسبة 95 % عند نفس مستوى التدفق لغاز الارجون.

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1. Introduction

As the name suggests, plasma denotes an ionized gas that contains electrons, ions, and neutrals, resulting from detachment of electrons from atoms or molecules; in contrast to gases, plasmas have their own characteristics, the stricter definition is that plasma is "a quasi-neutral gas of charged and neutral particles which exhibits collective behavior"[1].

Coulomb forces, which are long-range and cause distant regions to interact, are responsible for plasma's collective behavior. One separates the elastic collisions, which do not modify neutral species' internal or potential energy but convey a weak rise in their kinetic energy, as a plasma state from neutral gas and other ionized gases. It is possible to use low-temperature plasmas to handle heat-sensitive materials like polymers and biological tissues, resultant to their inelastic collisions and relatively low ion and neutral temperatures, creating the first excited states of atoms and molecules[2,3].

Plasma needles' ability to process delicate surfaces and reach microscopic depths, combined with the fact that they operate at room temperature and under normal air pressure, makes them an attractive option for biomedical applications[4,5]. As it is able to remove bacterial contamination topical cells without causing any necrosis in neighboring cells during treatment.

The applied electric field from the employed power supply accelerates free electrons between the electrodes. These electrons head-on collide with gas molecules resulting in excited molecules and free roots in addition to electron-ion pairs.

When recharging electrodes stationed by the ability of the power supply, this ability works to accelerate free electrons between the electrodes, these electrons collide, head-on collisions, with gas molecules resulting in excited molecules and free roots in addition to electron-ion pairs. As these come out from the discharge chamber they re-combine with each other, but the flow remains to include a semi-stable element. These atoms or molecules of the argon gas in the excited state have an excited lifetime longer than the existing lifetime in case of excited normal, but generally, the lifetime in the excited state is smaller than the lifetime in the ground state during the non-thermal plasma generation. Free radicals formed during plasma generation are described as atoms or molecules with non-marital electrons or contains an open casing. In addition, some ultraviolet radiation is generated when plasma is formed [6,7,8].

Oxygen is employed by most creatures to breakdown food and obtain energy through a process known as respiration. Aerobes are organisms, as are most bacteria, that utilize oxygen for respiration. On the other hand, Anaerobes are organisms that have evolved to live without oxygen [9].

The cell wall of both gram negative and gram positive cells contains peptidoglycan, which is responsible for protecting the cell. The peptidoglycan layer of the gram negative cell wall is very thin and often comprises only 10% or less of the cell wall, while the gram positive cell wall has a peptidoglycan layer approximately comprising 90% of the cell wall, thus providing these bacteria with higher strength and rigidity, making them harder to be sterilized[10,11].

Peptidoglycan serves as an intermediary layer between the two membranes of the gram-negative cell envelope (cytoplasmic and outer). Lipopolysaccharide and other lipid and protein components are found in the outer membrane. The spore-forming ability of gram-negative cells is not present. To be clear, gram-negative bacteria are no less pathogenic than

their gram-positive counterparts because they cannot form spores. Figure 1 depicts the cell wall composition of gram-positive and gram-negative bacteria [12].
The aim of this research is to expose these bacteria to non-thermal plasma and study the effect of distance between the bacteria sample and plasma plume, in addition to the exposure time required for killing the bacteria sample with all the variables that were used during the treatment.

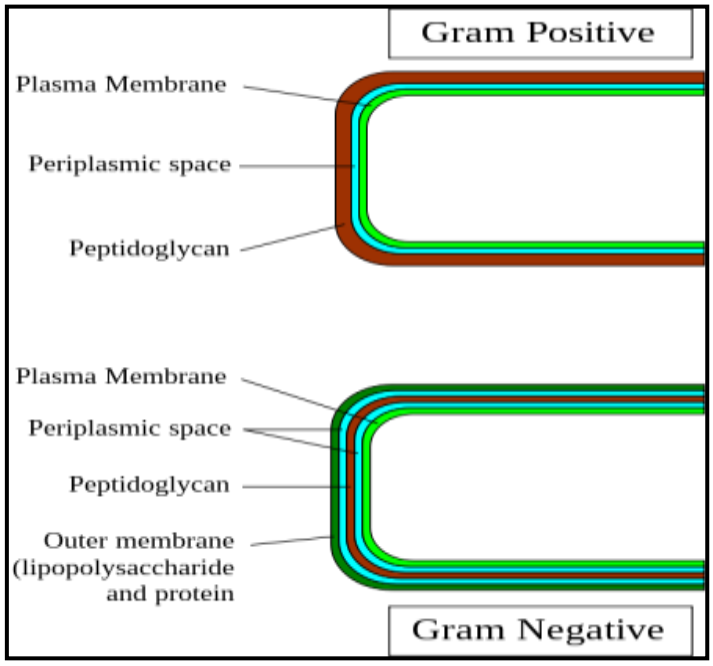

Figure 1: Cell wall composition of gram-positive and gram-negative [8]

2. Experimental Part

In this work, non-thermal plasma system was prepared to be used to expose the *Pseudomonas aeruginosa* bacteria to plasma. This includes argon gas with a flow device, an AC high-voltage power supply (1-20 kV of 5 KHz frequency) and isolated samples of bacteria. The inner electrode tube of the non-thermal plasma system is made of steel; it participates in the ionization processes and withstands high voltage and high temperatures. The outer tube surrounding this steel tube is made of Pyrex glass (which as resistant material), and the outer electrode is made of copper and connected to a high-voltage power supply, which is placed at the front of the needle tube, as shown in Figure 2.

Inoculated *Pseudomonas aeruginosa* bacteria not exposed to non-thermal plasma serve as the control group. After exposing the bacteria isolate to non-thermal plasma, they were planted in petri dishes containing media special for each bacteria, and placed in an incubator at 37 °C for 24 hours, from which 1.5 x 10^8 (CFU/ml) of bacteria were extracted as determined by 0.5 McFarland standard. After incubation, the colony forming units (CFU) were counted in order to check the efficiency of the non- thermal plasma needle system on bacterial inactivation.

The bacteria isolates were taken from Al-Yarmouk Teaching Hospital / Department of wounds and burns-microbiology laboratories, after being diagnosed according to biochemical and morphological tests.

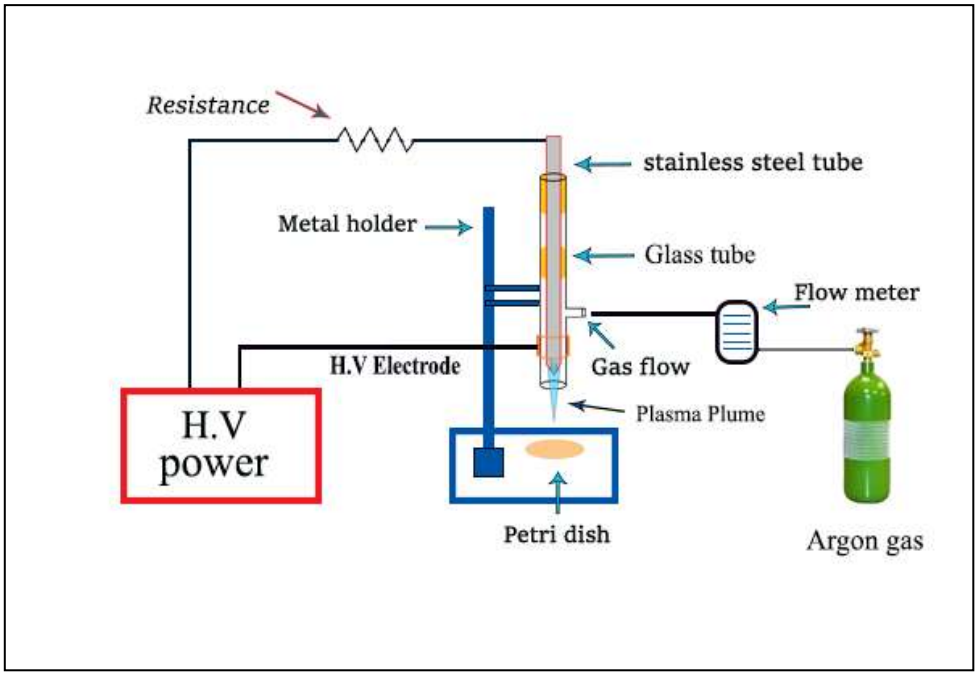

Figure 2: Production system of non-thermal plasma.

The bacterial isolates were placed in petri dishes to be exposed to the non-thermal plasma for different times of (1-5 min.). The effect of different petri dishes-plasma needle distance, gas flow rate, and applied voltages were also studied, as shown in Figure 3.

Figure 3: *Pseudomonas aeruginosa* bacteria Isolate in Petri dish during Exposure to Plasma.

3. Results and Discussion:

Figure 4 shows the effect of different argon gas flow rates of (1-5) *l/min* on the killing efficiency of plasma at a distance of two cm and at two values of applied voltage (4.9kV and 8kV) for different exposure times. More than 52% of the bacteria were killed at a gas flow

rate of 1 *l/min* and 4.9kV. The *Pseudomonas aeruginosa* bacteria killing rate was more than (91%) as the gas flow rate was increased to (5 *l/min*) at (5min) exposure time.

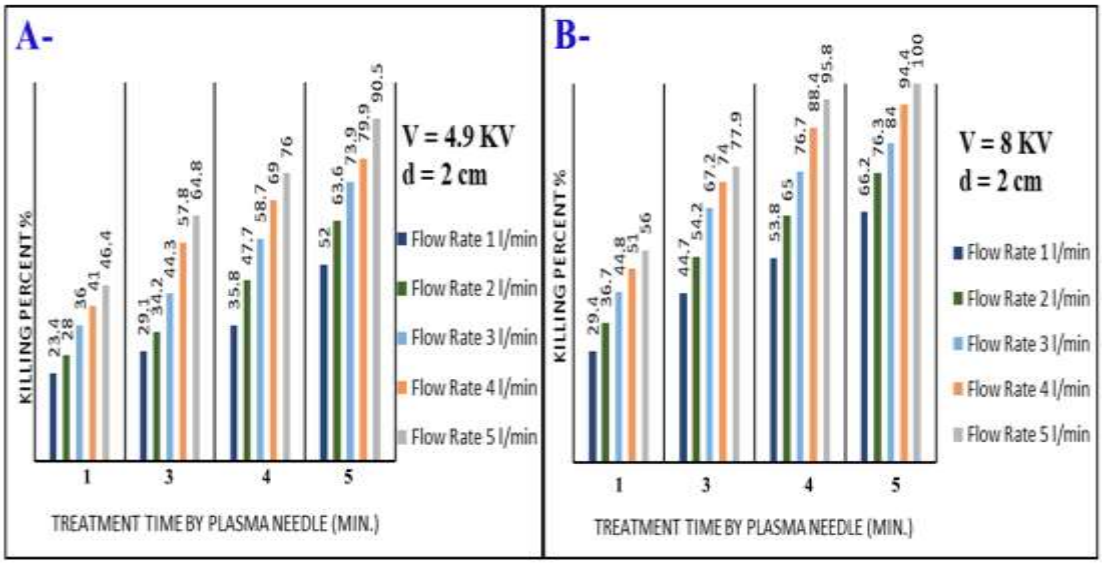

Figure 4: The percentage killing of *Pseudomonas aeruginosa* bacteria as a function of different gas flow rates at distance (2cm) at (A) 4.9 kV and (B) 8 kV.

Results revealed that increasing the gas flow rate at a voltage of 8kV led to an increase in the killing rate of *Pseudomonas aeruginosa* bacteria by 66%, resulting in in 100% kill rate of (Pseudomonas) bacteria at 5 *l/min* gas flow rate.

Additionally, the particular effects of high voltage and high-velocity particle discharge piercing through the bacterium's exterior structure are crucial to plasma's ability to inactivate the bacteria. Because of the high-speed particle discharge, the cell membrane's structure and charge distribution are destroyed, leading to the bacterium's death. Because the spore's exterior shell was tighter than that of the vegetative form, plasma was able to break the vegetative form but not the spore [13].

However, the rise of the gas flow rate led to an increase of ionizations and thus increase in the formation of active roots, such as ozone O_3, hydrogen peroxide H_2O_2, and hydroxyl radicals. Also, ROS and RNS were generated in the plasma such as superoxide, atomic oxygen, hydro peroxyl radicals, nitrogen oxides, nitrites, nitrates, peroxy nitrites and UV radiation. This would greatly affect the killing of the bacterial colonies, UV radiation penetration and diffusion of plasma-generated active species are hindered, when H_2O_2 is formed, plasma needle becomes a powerful oxidizer and an active tool for the inhibition of bacteria. It has an immediate effect on the outer membrane of bacteria because of the peroxidation of cell membranes. With increasing the exposure time, the number of bacteria colonies decreased it has already been reported that certain chemotherapeutic agents and radiation therapies cause oxidative stress by enhancing ROS in patients when used as a cancer therapy. When the amounts of ROS rise to the toxic threshold level, the antioxidant system of the cell is eventually altered, possibly leading to cell death. In this scenario, the death of cancer cells can be increased by using exogenous ROS-generating agents, because they cause enhanced ROS stress. Oxidative stress can induce many biological responses, The extent of these responses can depend on the cellular genetic background, the different classes of specific ROS involved, and significantly on the intensity and duration of the oxidative stress created [14,15,16].

However, the results differed when the distance between the isolated bacteria sample and the plasma needle was increased to 3 cm at the same voltages (4.9kV, 8 kV). The effect of the distance was noticeable and led to a decrease in the killing rate of (*Pseudomonas aeruginosa)* bacteria. The results indicated different rates in the killing of *Pseudomonas aeruginosa* bacteria during different gas flow rates. At a voltage of 4.9 kV, the rate of killing bacteria was more than (50%) at a gas flow rate of (1*l/min*) for (5min) exposure time, while the rate of (*Pseudomonas aeruginosa)* bacteria killed was more than (88%) when the gas flow was increased to (5 *l/min*) through the same exposure time of (5min). Increasing the voltage to (8kV), the effect of the distance was apparent, in the lack of a complete kill ratio compared to the isolates that were exposed to the non-thermal plasma at a distance of (2cm) with the same voltage. This indicates the positive relationship between the distance of the plasma nozzle and the killing of bacteria (*Pseudomonas aeruginosa)*. Figure 5 shows the killing rate as function of exposure time of bacteria (*Pseudomonas aeruginosa)* to plasma at (3cm) distance and (4.9kV and 8kV) voltages.

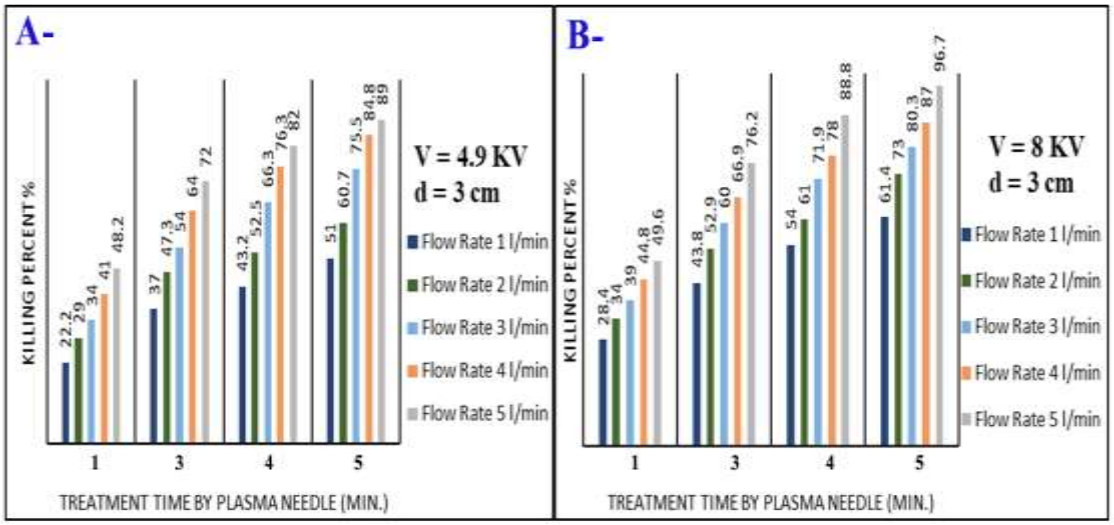

Figure 5(A-B): Shows the killing percent as a function of different flow of gas on bacteria *Pseudomonas aeruginosa* at the voltage (4.9 - 8 kV) and distance (3cm).

The best exposure time to kill the gram-negative bacteria (*Pseudomonas aeruginosa)* was (5min), with a (8kV) voltage, at (2cm) bacteria sample- plasma tip distance, and (5*l/min*) gas flow rate. Figure 6 shows an image of the control bacteria not exposed to non-thermal plasma, and images showing the different killing rates of *Pseudomonas aeruginosa* bacteria at different times of exposure to non-thermal plasma; the images show different proportions of killing because of the resistance of the bacterial cell wall to non-thermal plasma [17].
The last image (4-14D) shows the complete killing of bacteria after five minutes of exposure to non-thermal plasma.

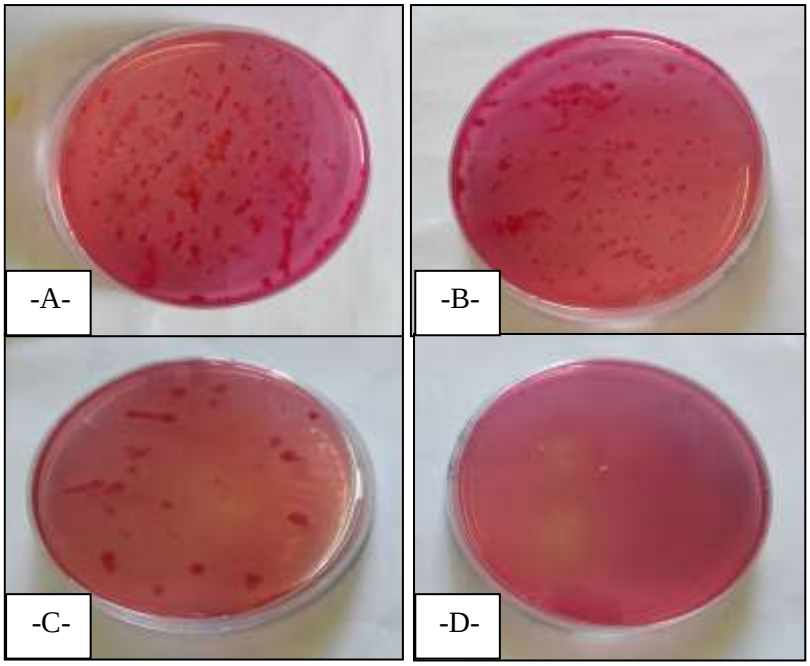

Figure 6: *Pseudomonas aeruginosa* isolates through exposure to non-thermal plasma system. A: Control, B: 1 minutes, C: 4 minutes, D: 5 minutes.

Moreover, the general behavior of the current-voltage in this system is not very different at all gas flow velocity and is somewhat similar since the current values are of very few microamperes which recorded a slight increase with the increase of voltages up to reach a certain value and then happens the so-called breakdown voltages. This value depends on the velocity of the argon gas flow, and once the breakdown voltages occur, the plasma is produced by the needle, with different intensity and size depending on the velocity of the gas flow. Also, it was found that the values of the current increase at the breakdown voltage to a certain value that varies according to the velocity of gas flow, and current values continue on this case with a slight decrease when increasing the voltages [18]. All these factors enhance the effect of distance on killing gram-negative bacteria and destruction of the cell membrane, resulting in the release of cytoplasm and cell death. This can be explained, that when the voltages are low (less than the breakdown voltages), the energy processed to the molecules by the electric field is low and is incapable of producing elementary and secondary ionizations to molecules, and by increasing voltages, begins the initial ionization of the molecules and register low values for the current [19].

4. Conclusions

The previous results showed when exposing bacteria to non-thermal plasma at a variable flow rates of argon gas, as well as with a change of the distance between the sample and the plasma nozzle, a clear effect on the percentage of bacterial killing was noted. Better bacterial killing was obtained at a distance of 2 cm than at 3 cm distance. The main reasons for this includes the destruction of the protein layer and the rupture of the living membrane of bacteria. It is possible to consider this study to know the effect of non-thermal plasma at atmospheric pressure or what can be called cold plasma on bacteria isolated from patients with burns for the purpose of using plasma in medical and biological treatment to accelerate the healing of wounds and burns.

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