

## The influence of argon gas flow in the killing of staphylococcus epidermidis bacteria

Ahmed Mahmoud Shihab<sup>1</sup>, Hamid H. Murbat<sup>2</sup>, Mohammed Ubaid Hussein<sup>3</sup>,  
Salam Dawood Muhamed<sup>1</sup>

<sup>1</sup>Department of Physics, College of Science, Anbar University

<sup>2</sup>Department of Physics, College of Science Women, University of Baghdad

<sup>3</sup>Department of Physiology and Medical Physics, College of Medicine, Anbar  
University

E-mail: ahmedmsh049@gmail.com

### Abstract

In this research, non-thermal plasma system of argon gas is designed to work at normal atmospheric pressure and suitable for work in medical and biotechnological applications. This technique is applied in the treatment of the Staphylococcus epidermidis bacteria and show the role of the flow rate of Argon gas on the killing rate of bacteria, and it obtained a 100 % killing rate during the time of 5 minutes at the flow Argon gas of 5 liters/ min.

### Key words

Non thermal plasma, plasma needle, argon gas, Staphylococcus epidermidis bacteria.

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## تأثير تدفق غاز الأرجون على نسبة قتل بكتيريا Staphylococcus epidermidis

احمد محمود شهاب<sup>1</sup>، حامد حافظ مربوط<sup>2</sup>، محمد عبيد حسين<sup>3</sup>، سلام داود محمد<sup>1</sup>

<sup>1</sup>قسم الفيزياء، كلية العلوم، جامعة الانبار

<sup>2</sup>قسم الفيزياء، كلية العلوم للنبات، جامعة بغداد

<sup>3</sup>قسم الفسلجة والفيزياء الطبية، كلية الطب، جامعة الانبار

### الخلاصة

في هذا البحث، تم تصميم منظومة البلازما غير الحرارية تعمل بغاز الأرجون عند الضغط الجوي الاعتيادي ومناسبة للتطبيقات الطبية والبايوتكنولوجية. حيث تم تطبيق هذه التقنية في معالجة بكتيريا ستافيلوكوكاس ابيديميس وتبيين دور معدل تدفق غاز الأرجون على معدل قتل البكتيريا، وحصلنا على معدل قتل بنسبة 100% خلال زمن 5 دقائق عند تدفق 5 لتر/ الدقيقة.

### Introduction

Plasma is defined as the fourth state of matter. The other states of the substance are solid, liquid and gas. The fourth state, plasma, is ionized gas. Most of these substances in the universe is plasma. It is used in

industrial and in medical applications. In the industry the plasma are used in many technologies: plasma television screens, light systems and power systems. A relatively new area is plasma use in biomedical applications and dentistry [1].

There are several ways to generate non-thermal plasma. Energy is needed to produce and sustain plasma. This can be done in several ways: through thermal, electrical, or photovoltaic energy. Usually a gas discharge occurs electrically [2].

Electrons and ions can get energy from the applied electric field. When these particles are in minority, heating of neutral molecules will be limited. Thus, diffuse plasmas where the fraction of ionized species is below 0.1 %, are usually non-thermal. This situation is readily achieved under reduced pressures, in the range of 10 to 1000 Pa, which keeps the charge density low. Moreover, the frequency of elastic collisions between electrons and molecules is low, so electrons do not have much chance to convey their energy to the gas [3, 4].

The temperature of the electron is usually greater  $10^4$  °K, while the temperature of both neutral particles and ions depends heavily on the type of plasma produced temperature can vary from approximately room temperature to  $10^7$  °K. It is usually for each class of plasma components of its own degree the temperature of the  $T_e$  electrons and the positive ions of  $T_i$  and  $T_n$  neutral molecules. So it can be said that plasma is the only substance that contains several temperatures at the same time [5].

In this type of plasma ions and the temperature of neutral particles surrounding the same, the electron temperature rises much higher than any  $T_e \gg T_i \gg T_n$  in the cold plasma, most of the processing energy in the electrons in the plasma, this produces effective electrons instead of gas heating as a whole, because ions and neutral components remain relatively cool. This feature will enable us to use plasma to process sensitive materials, including biological tissue [6].

### Staphylococcus epidermidis bacteria

This type of bacteria is a type of gram positive bacteria were first discovered in 1880 in Aberdeen, Scotland, by surgeon Alexander Oxton when he isolated bacteria from pus in the knee joint. Frederick Julius Rosenbach later named the bacteria by that name [7]. Although they are often considered human microbes, they can sometimes cause diseases. Specifically, Staphylococcus epidermidis is one of the most common causes of bacteremia and endocarditis. In addition, bacteria can cause numerous infections in the skin and moist tissues, especially when mucous tissue lesions occur, which can be found naturally on the surface of the skin and in the nose, they also live naturally in the lower reproductive tract of women [8].

### Experiment setup

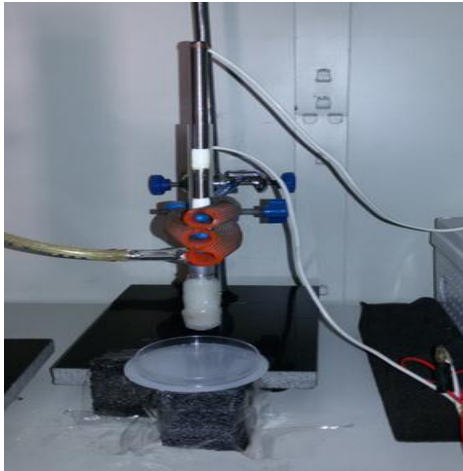
#### Plasma torch

Non-thermal plasma needle is designed where the device is a tube on cylindrical shape, inner diameter of 1 cm and a length of 18 cm are made of Pyrex thickness 1.2 mm, open on both sides and has a slot width 3 mm from 4 cm to the top of the tube allows to the flow of argon gas.

The inner electrode inside of the tube is made of stainless steel metal diameter of 8 mm and a tapered in front and a length 20 cm, elongated inside the tube and isolated by teflon, making a distance 5 mm at the end of the tube from the slot of the needle so as to obtain the discharge process at the end of a tapered tube with gas flow which passing through this tube, and connects this electrode with cathode of power supply. The other electrode positioned from the outside to the top of the tube and made of copper, which is a slice with thick 0.5 mm and width 2 cm where it was connects this

electrode with anode of a.c power supply.

Then the silicon insulator was placed on the outer Aluminum electrode, so as to prevent direct discharge occurs along the tube, Fig.1 shows the non-thermal plasma needle.

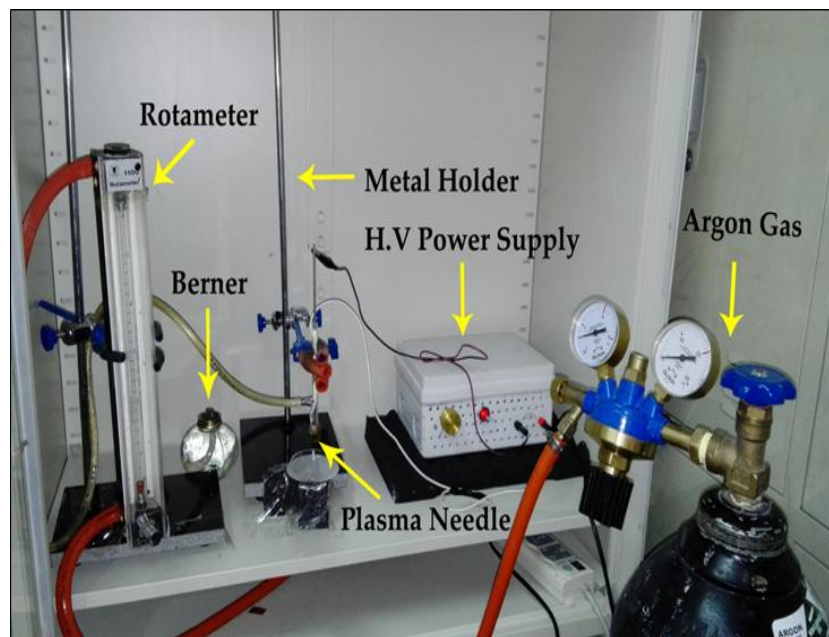


**Fig.1: The non-thermal plasma needle system.**

## Method

The *Staphylococcus epidermidis* bacteria were obtained from the Yarmouk Teaching Hospital from the Central Education Laboratories, which were isolated from medical devices and transferred by Dash Police to the Central Environmental Laboratory at the University of Baghdad.

To prevent contamination during the experiment, the plasma system was placed in isolation from the laboratory chamber as shown in the Fig.2 and it was sterilized with 70 % alcohol, the temperature of the bacteria in the distilled water and the suspension of the bacteria in Petro dish and placed under the system and exposure to the plasma at specific times from 1 to 6 min.



**Fig.2: Image of non-thermal plasma needle system.**

After exposing these samples to plasma, they incubated for 24 hours at 37 °C. After the end of the incubation period, bacterial colony formation units were calculated in a CFU method in order to verify the efficiency of killing the bacteria using the non-

thermal plasma system, the killing effect of plasma system on both *Staphylococcus epidermidis* bacteria isolates were analyzed using a killing efficiency equation as follows [9]:

$$\text{Killing efficiency} = \frac{(N_0 - N_t)}{N_0} \times 100\% \quad (1)$$

where

$N_o$  = CFU of non-treated bacteria (control).

$N_t$  = CFU After treatment of bacteria.

## Results and discussions

### Influence of argon gas flow rate on the Bacteria deactivation

When the distance between the sample and the system nozzle is

installed at a distance 2 cm, the diameter of the tube is 10 mm and the voltage is 5 kV a.c for different flows 1, 2, 3, 4, 5 l/ min. The results showed that the rate kill of bacteria increases with increasing the time of exposure to plasma, it increases with increased flow of argon gas, as show at Fig. 3.

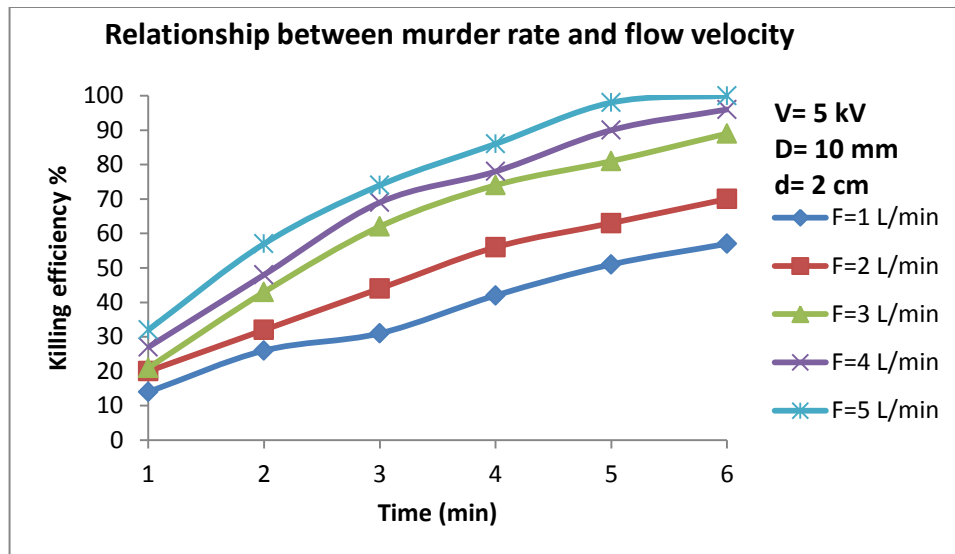


Fig.3: The rate of killing bacteria as a function of time for different gas flow rate.

Fig. 3, shows the same behavior, when increasing the exposure time the plasma increases rate of killing of bacteria, as well as the flow rate of argon gas has an effect on rate of killing bacteria, because the number of gas molecules passing through the plasma tube increases, which in turn increases plasma density. Where the highest kill rate is 100 % according to Eq. (1) at the flow rate 5 liters / min and time 5 minutes.

The results show that the flow rate of argon gas was 1 liter/ minute in the first minute. The bacterial killing rate was 14 %. In the 5th minute, the bacterial killing rate was 57 %. When the flow rate of argon gas was 5 liters/ minute in the first minute, the bacteria killed were 36 %. In the 5 min.; the bacterial killing rate was 100 %. This indicates that the percentage of

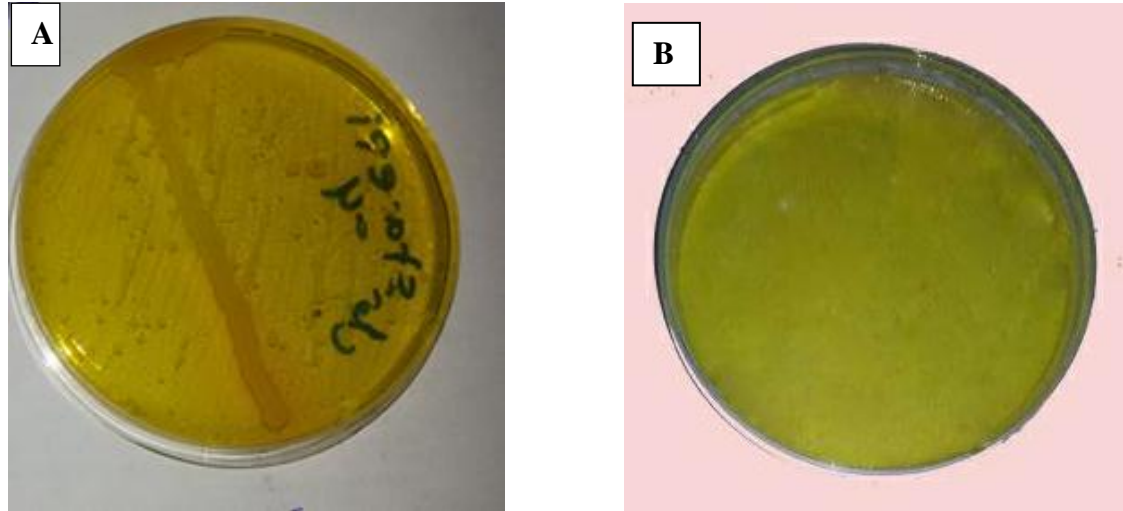
bacteria killed increases with the time of exposure to the plasma and increase the flow rate of argon gas.

Effects of increased gas flow rate and discharge of high-velocity particles penetrating through the outer structure of bacteria may play a dominant role while disabling bacteria caused by a plasma needle. If the bacteria's is treated increase the flow rate of gas, the structure of the cell membrane and electrical distribution of charges on the cell membrane can be destroyed. In addition with the penetration effect of high-speed particle discharge the external structure of the bacteria, they can destroy the cell wall, the cytoplasm will release, which will cause the death of bacteria. Because the exogenous structure of the spore was more rigid than that vegetative form, vegetative form can

be divided by plasma and spore cannot be broken but only left with cuts by plasma needle [10].

Fig. 4 shows the best results of the rate of killing of bacteria when

exposed to plasma at the flow of 5 liters/ minute and for different periods of time, the highest killing rate was 100% at 5 minutes.



**Fig.4:** *Staphylococcus epidermidis* bacteria growth samples after exposure to plasma jet system:- **A:** control , **B:** after treatment of bacteria.

The influence of argon gas flow rate on the bacteria deactivation, the effects of the increasing gas flow rate and high speed particle discharge penetrating through the outer structure of the bacteria may play a dominant role during the inactivation of the bacteria caused by plasma needle. at the operating condition that applied in this work, the inactivation time for the *Staphylococcus epidermidis* bacteria at time 4 min of same conditions [11].

### Conclusions

Non-thermal plasma needle is designed to work at atmospheric pressure. The rate of the kill depends on the operating conditions of the plasma system such as gas flow, distance between tip of the needle isolated bacteria and time of bacteria exposure to plasma.

Effect of flow rate of argon gas on killing bacteria, the effects of increased gas flow rate and discharge of high-velocity particles penetrating through

the outer structure of bacteria may play a dominant role while disabling bacteria caused by a plasma needle, the time of killing bacteria was 5 minutes.

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