The role of atmospheric non-thermal plasma in the bacteria inactivation

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Abstract

Non-thermal atmospheric pressure plasma has emerged as a new promising tool in medicine and biology. In this work, A DBD system was built as a source of atmospheric pressure non-thermal Plasma suitable for clinical and biological applications. E. coli and staphylococcus spp bacteria were exposed to the DBD plasma for a period of time as inactivation (sterilization) process. A series of experiments were achieved under different operating conditions. The results showed that the inactivation, of the two kinds of bacteria, was affected (increasing or decreasing) according to operation conditions because they affects, as expected, the produced plasma properties according to those conditions.

Key words

Atmospheric non-thermal plasma, sterilization, E. coli, Staphylococcus spp.

Article info.

Received: Nov. 2014
Accepted: Jan. 2015
Published: Apr. 2015

دور البـلازمـا غير الحـراريـة فـي تعطـيل البكـتـيريـا

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الخلاصة

تعد البلازما غير الحرارية من الأدوات الحديثة في الطب والبايولوجي. تم في هذا البحث بناء منظومة تفريغ كهربائي خلال حاجز عازل كمصدر للبلازما اللاحرارية في الضغط الجوي الاعتيادي وهي مناسبة للتطبيقات الطبية والاحيائية. وقد تم تعريض نوعين من البكتيريا لبلازما تفريغ الحاجز العازل كعملية تعطيل (تطهير) للبكتيريا. هذا وقد انجزت سلسلة من التجارب تحت ظروف تشغيلية مختلفة وبينت النتائج ان عملية التطهير، لكلا نوعي البكتيريا، تتأثر (زيادة او نقصان) تبعا للظروف التشغيلية لانها تؤثر، كما هو متوقع، في خصائص البلازما المنتجة تبعا لتلك الظروف.

Introduction

Plasma in physics is the ionized state of matter and it is the most of universe matter, as fire in the sun, stars, etc... [1]. Plasma consists of positively and negatively charged ions, electrons and neutral species (atoms, molecules). It was divided into two types; hot and cold plasmas. Hot plasma or non-equilibrium plasma consists of very high temperature particles and they are close to the maximum degree of ionization, while cold plasma composed of low temperature particles and relatively high temperature

electrons and they have a low degree of ionization [2].

Recently there has been increased interest and development in cold plasma processes working at atmospheric pressure by the growing requirements of new plasma technology that can allow continuous plasma processing, like plasma needle [3, 4], the hair line plasma [5], and micro capillary plasma jet [6]. Also, cold plasma is used in many areas such as, surface modification of polymers [7], sterilization [8], and inactivation of bacteria [9].

The dielectric barrier discharge (DBD) is most frequently used as a non-thermal plasma source that can be operated with different gasses at elevated pressures (up to atmospheric pressure) [10, 11]. The plasma is created between two conductive electrodes connected to an AC or pulsed power source. At least one of the DBD electrodes is covered by a dielectric layer, which prevents the arc formation after breakdown.

DBD discharge usually consists of a large number of short-living micro channels (filaments) that are randomly distributed over the entire area of the dielectric barrier. Despite a high breakdown voltage in gas at atmospheric pressure (several kV); the average electric current is low. Therefore, DBD plasma can be applied directly to living tissue and open injuries without causing them damage [12, 13].

Escherichia coli (E. coli) are a classic opportunistic pathogen found in hospitals. The World Health Organization professes that this bacterium is one of the primary pathogens of hospital acquired infection [14]. *E. coli* contributing to a large percentage of nosocomial infections ranks first in the infection rate of various gram-negative nosocomial pathogens [15, 16]. In recent years, because of the multi-drug resistant mechanism of *E. coli*, infection incidents have occurred frequently, and the drug-resistance of the bacterium has gradually risen [17]. On blood agar, the colonies appeared 3-4 mm in diameter, on MacConkey agar; the colonies were red, since this organism is a lactose fermenter [18, 19].

Staphylococcus spp is a spherical gram-positive bacterium that tends to form irregular colonies; some cause boils or septicemia or infections. The size, shape and arrangement of bacteria, and other microbes, is the result of their genes and thus is a defining characteristic called morphology [19,20]. Gram stained smear from such colonies showed gram positive cocci with grape like clusters [18]. On blood agar, colonies are 2-4 mm in diameter after 18 hours incubation at 37^0C, smooth, shiny, opaque, yellow or white domes resembling small drops of glass paint. Medium in size, dome shaped, creamy with beta haemolysis on blood agar [19].

Conventional electric discharges are well-known for their ability to sterilize surfaces. The advantage of DBD plasma treatment is its ability to sterilize living animal or human tissue without any damage. Ultimate use of DBD plasma is to treat wounds of human patients, so our sterilization tests were performed by swabbing and culturing bacteria from human skin samples before and after DBD plasma treatment.

Experiment setup

Dielectric barrier discharge DBD system is based on a conventional dielectric barrier discharge. It is basically a system driven by alternating current and high voltage applied between two conductors where one or both are covered with a dielectric. That is to limit the current and to prevent transition to an arc. A simplified schematic of the DBD system setup is shown in Fig. 1.

The system consists of power supply of high voltage (5-25 kV) related to a wire to the copper electrode is surrounded by Teflon; the end of the copper electrode is connected in contact with the glass thickness of 1 mm. Other part of the system is gradually moving catcher connected by a piece of mica to the copper electrode to prevent the transmission of discharge to the catcher moving. Plasma is generated by applying alternating polarity or pulsed high voltage between the insulated electrode and the sample which must be treated. The sample putted on an aluminum substrate. A 1 mm thick polished glass was used as an insulating dielectric barrier. The discharge occurs between the bottom surface of the glass and top surface of the sample. The distance where the discharge occurs was controlled to be (1- 3 mm). To accomplish the control ability, the high voltage electrode was connected to a vertical catcher by a positioner. This positioner can be moved up and down easily. The diameter of the copper electrode employed was 2.5 cm. All the treatments are at room temperature and atmospheric pressure and were carried out according to the same procedure.

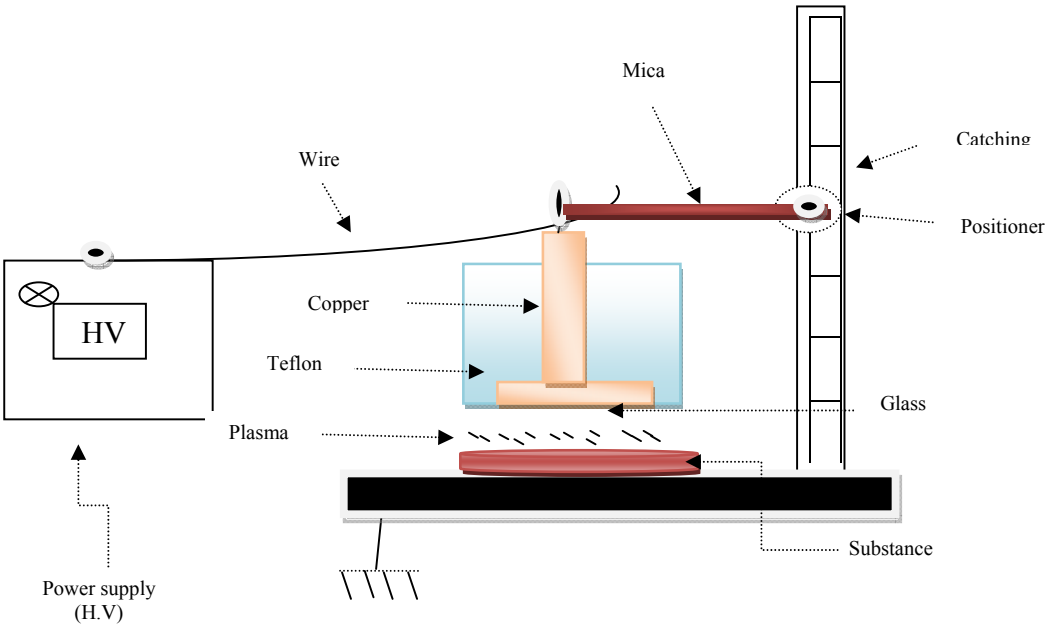

Fig.1: Schematic diagram for the DBD system.

Method

Twenty patients complaining from different clinical signs and symptoms were admitted to Ramadi Teaching Hospital for seeking medical advice was included in the present study. These samples were collected from patients visiting Teaching Hospital. The results of this work were obtained by studying 20 burns samples. They were 15 males and 10 females.

Sterilization tests were performed by swabbing samples from patient's skins. Bacteria (*E. coli*, *Staphylococcus spp*) were isolated from the samples and cultured using artificial media (MacConkey agar, Blood agar) to obtain bacteria colonies.

Sterilization was employed by exposing the bacteria to DBD plasma for a period of time. The prepared culture plates were treated by DBD plasma, and they are then incubated for (24h).

Results and discussion

For our samples, a large amount of bacteria were obtained from a swab skin patient on a selective media (MacConkey agar, blood agar). The prepared culture plates were treated by DBD plasma. The experiment was conducted in open air under atmospheric pressure and room temperature at 20^{o}C, and they are then incubated for (24h). There is no growth of bacteria on the area which treated by the DBD plasma for the period of time, as shown in Fig. 2. The figure shows that, while the plasma region diameter is roughly 2.5cm, the "inner" circle of inactivated of E. coli bacteria diameter is ~4.5cm and the "outer" circle where the bacteria is partially inactivated is ~5.5cm.

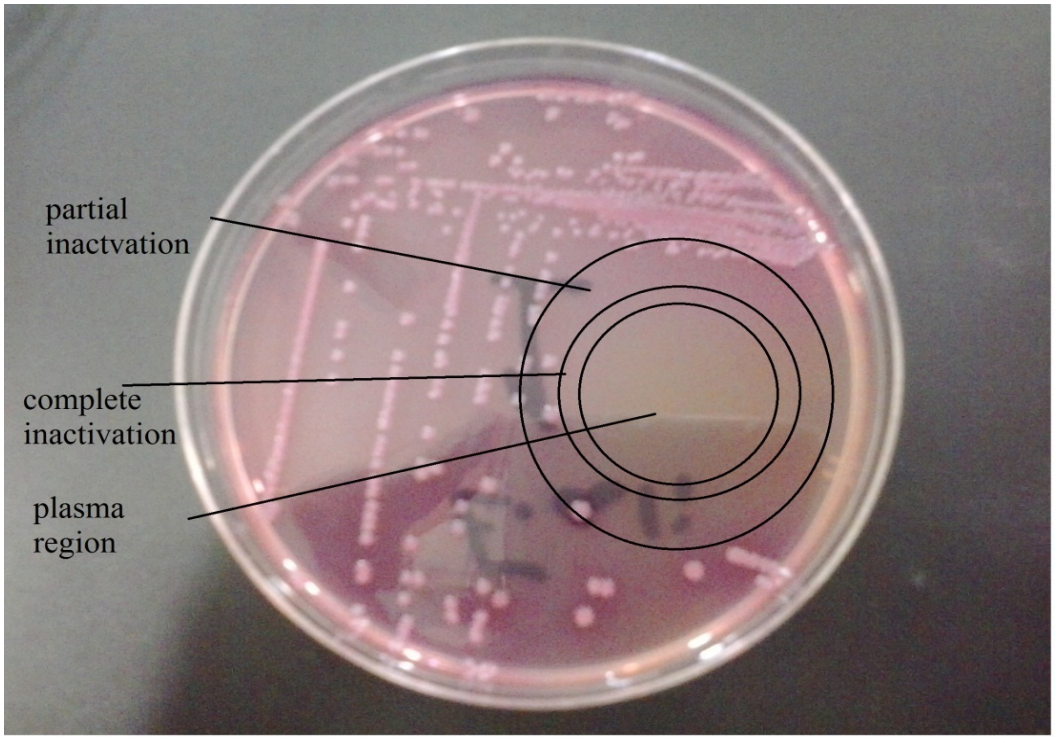

Fig.2: Petri dish with MacConkey agar after the DBD plasma treatment.

Fig. 3 shows the same above DBD plasma sterilization for bacteria cultured on a selective media is blood agar. Also, the figure shows, the plasma region diameter is roughly 2.5 cm, the "inner" circle of inactivated Staphylococcus spp bacteria diameter is ~4cm and the "outer" circle where the bacteria is partially inactivated is ~5cm.

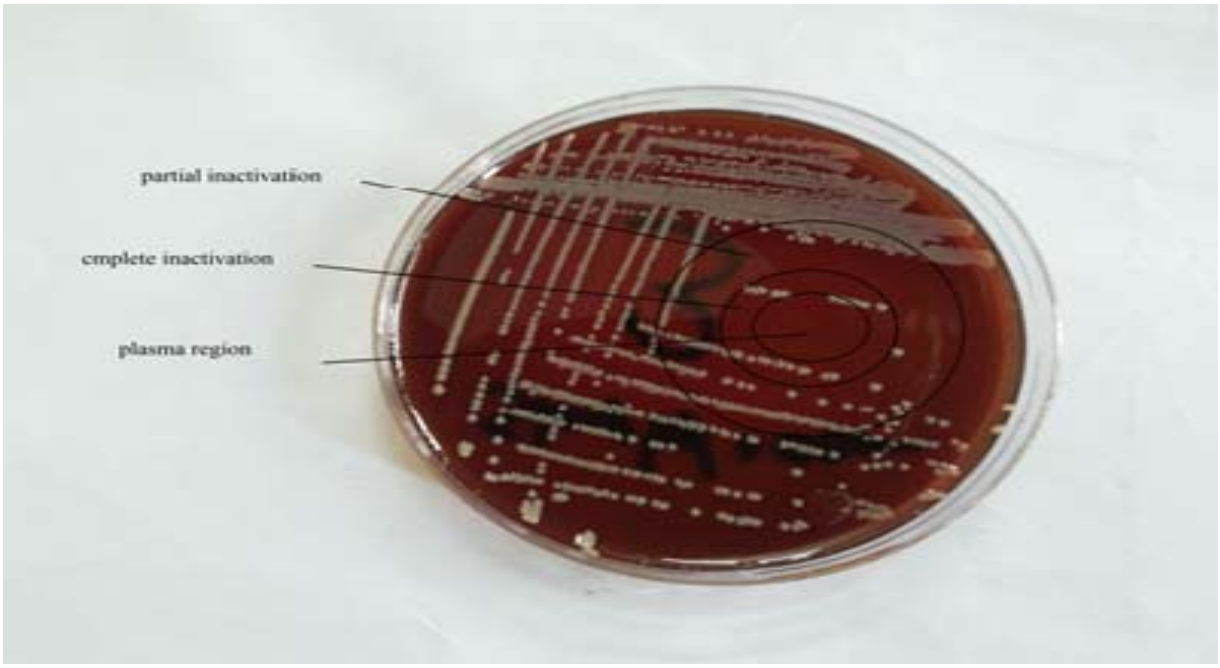

Fig.3: Petri dish with blood agar after the DBD plasma treatment.

Through (DBD) plasma at high rate the "complete inactivation " shifts only slightly and remains practically independent on the discharge rate employed ,even though further investigation is necessary ,these results suggest that direct treatment by plasma is more potent in bacterial activation than indirect treatment (by a jet for example). Hypothesize that this is due to high concentration of short-lived active species and radicals as well as UV radiation that is unable to penetrate far out of plasma region [21].

Taken some samples from bacteria (*E.coli* G- ve, Staph G +ve) to know the effects in/about plasma region, the applied voltage and distance between electrodes (gap distance) on sterilization process. Then the results of the sterilization by the DBD plasma for different bacteria types, under different operation condition of the DBD system, will be viewed and discussed.

E. coli Bacteria Sterilization

E. coli bacteria were sterilized by DBD plasma under the conditions of 17kV and 22kV applied voltages, and 1.5mm and 2.5mm gap distances. The results for complete and partial inactivations (sterilization) along a period of time were presented in Fig. 4. The figure shows, in general, the complete and partial inactivations diameters, for all voltages and distances, increase with the increasing of treatment time. Also, for each applied voltage, the inactivation (complete and partial) diameters increase with the decreasing of the gap distance. Then, a comparison between the (a) and (b) cases, in Fig.4, shows clearly the inactivation diameter increases with the increasing of the applied voltage. The inactivation diameter was varied due to the variation in the DBD plasma properties. The DBD plasma properties variation, as expected due to variation of the operating conditions such as the applied voltage and gap distance.

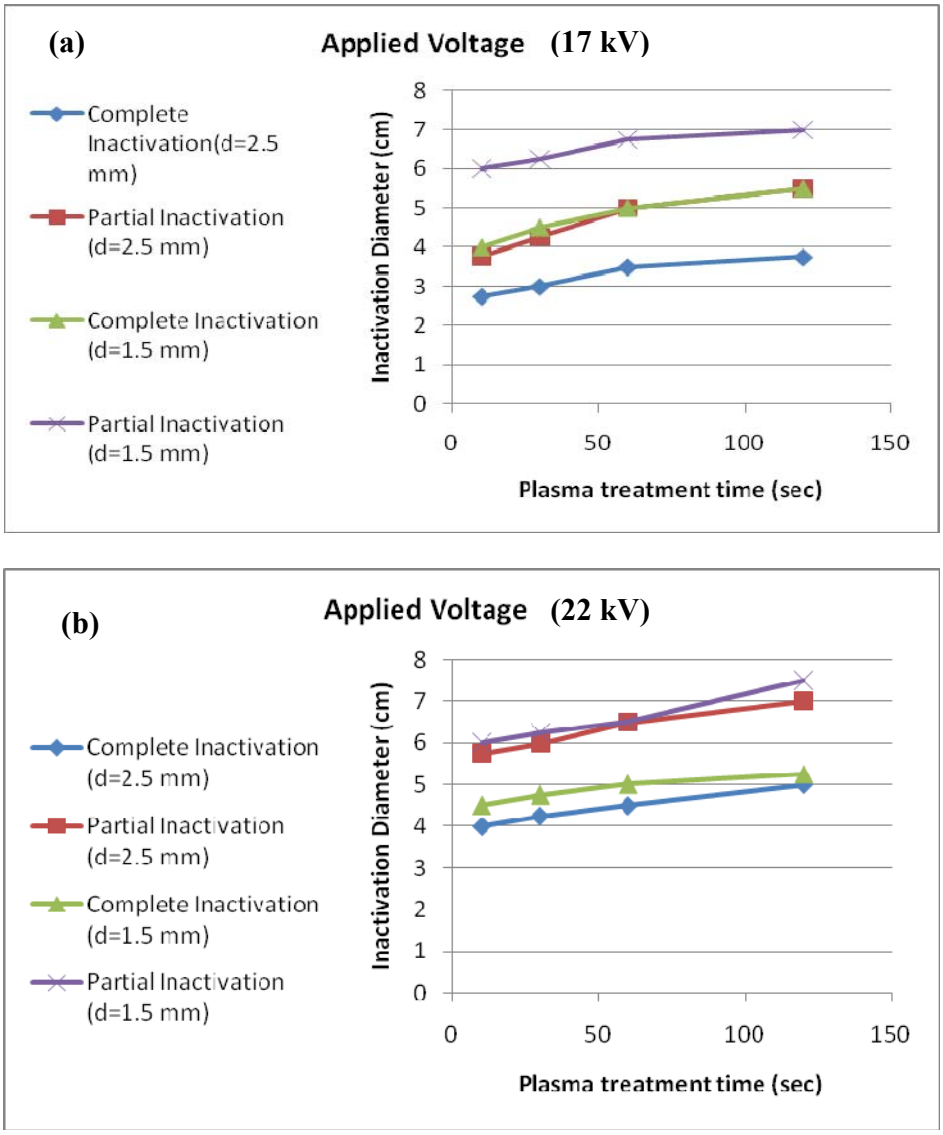

Fig. 4: The complete and partial inactivation diameter for E. coli bacteria as a function of the DBD plasma time treatment at 1.5mm and 2.4mm gap distance when the applied voltages are a) 17kV and b) 22kV.

Staphylococcus spp bacteria sterilization

As in the case of *E. coli* bacteria, the *staphylococcus spp* bacteria was sterilized by DBD plasma under the conditions of 17kV and 22kV applied Voltages, and 1.5mm and 2.5 gap distances. The results for complete and partial inactivations (sterilization) along a period of time were presented in Fig. 5.

Fig. 5 shows a same behavior for the complete and partial inactivation diameters. They increase along the treatment time and increase with the increasing of applied voltage and decreasing of gap distance. The important difference, one can observe, is the staphylococcus spp less sensitive to the DBD plasma treatment. In other words and for all conditions, Fig. 5 shows that the complete and partial inactivation diameters for the staphylococcus spp bacteria less than that for the E. coli bacteria at the same conditions.

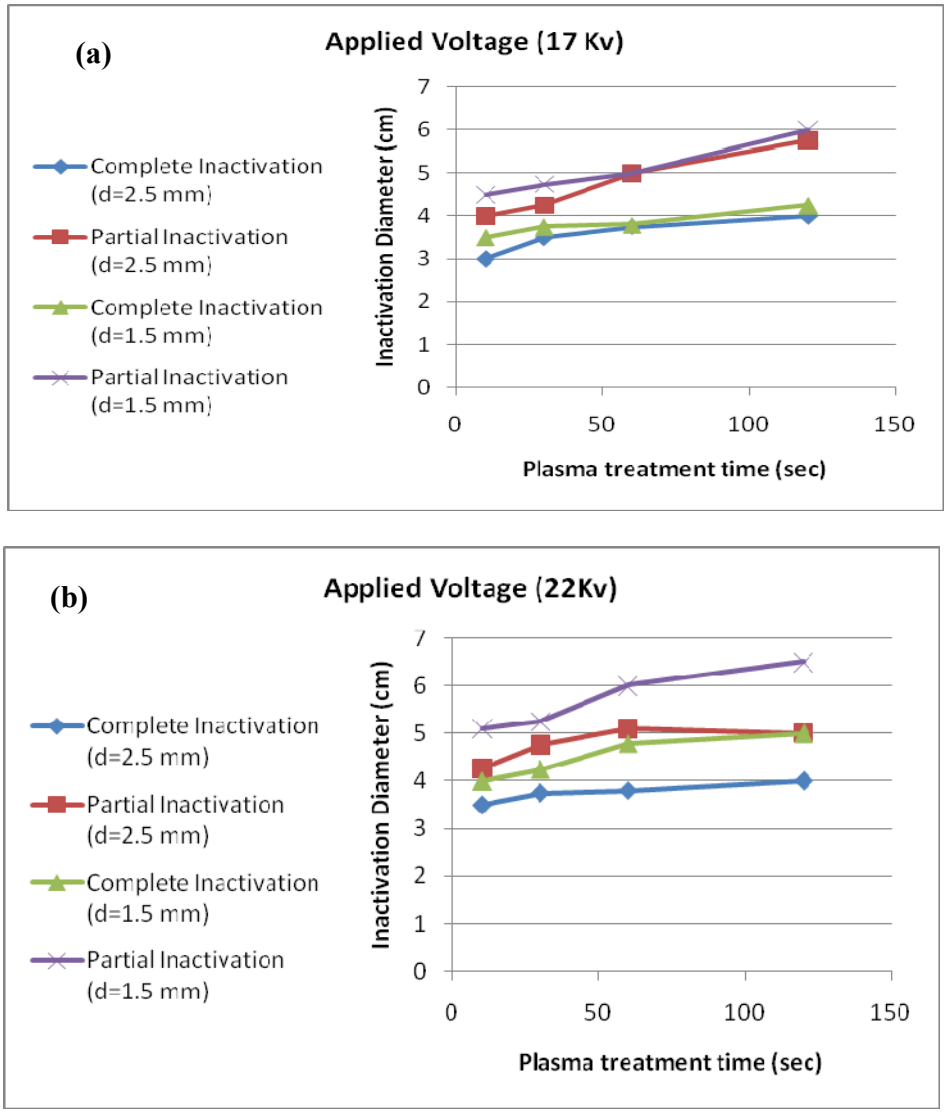

Fig. 5: The complete and partial inactivation diameter for Staphylococcus spp Bacteria as a function of the DBD plasma time treatment at 1.5mm and 2.4mm gap distance when the applied voltages are a) 17kV and b) 22kV.

There are several possible effects produced by the DBD plasma components on microorganisms, ions ,electrons and UV ray interact with complex process with cell together cause bacterial death.

Atmospheric non-thermal plasma was developed for sterilizing the *Staphylococcus spp*. The plasma was generated by dielectric barrier discharge (DBD), which was the sterilization of the bacteria showed that the cold plasma could effectively inactivate of *Staphylococcus spp* within 120 seconds and the sterilizing efficiency depended critically on the discharge parameter of the applied voltage [22].

The effects of the high voltage 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. If bacteria are treated with a high voltage electric field, the cell membrane's structure and electric charges distribution over the cell membrane can be destroyed. In addition with

the penetrating effect of the high speed particle discharge the outer structure of bacteria, namely cell wall and cell membrane of culture form, exosporium and coating of the spore, could be destroyed and cytoplasm would be released, which would cause the death of the bacteria. Because the outer structure of the spore was tighter than that of the vegetative form, the vegetative form could be broken by plasma and the spore could not be broken but only left with cuts by plasma [23]. However the sterilization efficiency drops off with increasing volume of liquid which inhibits UV penetration and diffusion of active species generated in plasma [24].

Conclusions

From the above results, one can conclude that:

- *E. coli* and *Staphylococcus spp* bacteria can be inactivated by exposed to the DBD plasma for a period of time. The inactivation increases with time increasing.
- The inactivation depends on the DBD system operating conditions such as applied voltage and gap distance; the complete and partial inactivation diameters increase with the applied voltage increasing and gap distance decreasing.
- *E. coli* bacteria more sensitive to the DBD plasma treatment than the staphylococcus spp bacteria; at the operating condition that applied in this work, the complete and partial inactivation diameters for the *E. coli* bacteria greater than that for the *staphylococcus spp* bacteria at same conditions.

Acknowledgments

We would like to express special words of thanks with deepest appreciation to Collage of Medicine and Clinical Laboratories in Al-Ramadi General Hospital, also the Staff working in these Laboratories.

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