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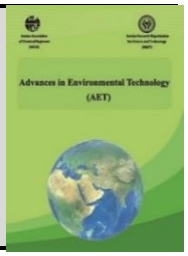

Assessment of anti-bacterial activity of non-thermal plasma in sterilization of infectious wastes

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ABSTRACT

In today's world, the production of hospital wastes and their adverse effects such as infectious outbreaks and resistance to treatment is an important issue. Therefore, it's vital to find a new and efficient method to manage such wastes. In this study, the ability of dielectric barrier discharge (DBD) plasma to deactivate *Pseudomonas aeruginosa* and *Staphylococcus aureus* bacteria was assessed. The bacteria were treated with DBD plasma after cultivation in liquid milieu, and then dried in a sterile air stream. The results showed that for both bacteria, the number of deactivated colonies increased proportionally to the time of treatment. First, it occurred rapidly, and then the number of active colonies decreased at a slower speed. Also, increasing the plasma duty cycle in the same treatment time led to more deactivated colonies. This increase was more significant in the *Pseudomonas aeruginosa* bacteria, and changes for *the Staphylococcus aureus* was slight.

1. Introduction

Waste disposal has become a major problem in recent years [1]. Although some waste contents are precious, societies are trying to find new ways to get rid of them [2-4]. Wastes can be classified into two different groups: solid waste and wastewater. Also, they can be sorted by their production source: regular, medical, industrial and special wastes. Medical grouping includes all the wastes produced by health protection units, research institutions and laboratories. In other words, all the infectious and hazardous wastes of hospitals, health and treatment centers, and medical laboratories are grouped in this category. Medical wastes are the main source of dangerous wastes for cities due to their high amount of pathological and medicine residues, chemical and radioactive substances, and used utensils. The technology needed for the aggregation, disposal, or recovery of these materials differs from municipal wastes and requires specific attention [5]. According to WHO medical wastes are sorted into eight groups of these includes infectious wastes, which consists of waste with the ability to spread at least one infectious disease. To avoid endangering patients, the sterilization of medical wastes is important. Sterilization is the process of annihilating all pathogenic and non-pathogenic micro-organisms such as bacteria and viruses in an environment. There are several methods applied to this issue: wet heat (autoclave), dry heat (oven), gamma radiation, ultraviolet radiation, chemical treatment, and, recently, plasma [6-8]. Plasma treatment is one of the most advanced methods for treating medical wastes. Plasma consists of different types: hot plasma with a temperature order of 4000 C has industrial applications; and cold plasma or non-thermal plasma that works by electrical discharge. Plasma treatment has gained attention for biological and medical uses due to its low temperature, independence of a big vacuum reactor, the existence of active species, and non-equilibrium nature [9-11]. Plasma is the same as an ionized gas. A plasma generating device produces an effective electrical conductor and a luminous matter like an

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electrical arc with high energy. This energy is transmitted to wastes as beam energy. One of the most efficient procedures to generate plasma is dielectric barrier discharge. This kind of cold plasma is formed in atmospheric pressure between two electrodes with at least one electrode covered by a dielectric like quartz or glass. Electric discharges happen by a high voltage alternative current, and the presence of the dielectric prevents high temperatures. It causes the production of ultraviolet rays and active chemical species like O and OH, which lead to the demolition of micro-organisms [12]. These benefits along with low temperature make it an excellent choice for items that are sensitive to heat like biological cells and tissues [13]. Bacteria are in a big group of prokaryote one-cell micro-organisms. Most have the length of several micrometers with different shapes such as spherical, cylindrical and springy. In regard to health, there are three types of bacteria: non-pathogenic, opportunistic, and pathogenic. The proliferation of bacteria is done by means of binary fission, blending, and budding [14,15]. Several works have been done to reduce or deactivate bacteria by means of DBD plasma. In previous research, DBD plasma was applied to *Escherichia coli* for about 70 minutes and destroyed them completely [16-18]. DBD also could be used in the peroxidation of *Escherichia coli* bacterium membrane fat [19]. In addition, DBD is an effective method in reducing the number of *Bacillus subtilis* bacteria [20]. Recent researches [21,22] show that DBD is a perfect tool for killing infectious bacteria. This powerful treatment presents an alternative for scientists to control dangerous infections and find a total solution for such diseases. In this study, the effect of DBD treatment on *Pseudomonas aeruginosa* and *Staphylococcus aureus* bacteria was investigated. The progress of bacteria destruction and its relation to the plasma duty cycle and the remaining time was shown. These bacteria were chosen because of their important role in pathogenicity and hospital infection issues.

2. Materials and methods

2.1. Plasma generation

For generating DBD plasma, a high voltage AC power supply with sinusoid was used. The maximum output voltage and power were 12 V and 30 W, respectively. The generator consisted of a 10-millimeter aluminum shaft which was insulated with a Teflon cover. The dielectric barrier was a disc with a 1-millimeter thickness and was connected to a high voltage electrode. The wire connected to the high voltage supply entered the Teflon cylinder from above and joined the high voltage electrode. The earth electrode was a stainless steel net, which was connected to the other side of the quartz disc. The plasma was produced near the earth electrode (steel net) and diffused outside. Finally, the samples were put near the probe (2 mm distance), so they could be treated by the plasma. A schematic view of the probe with a floating second electrode is shown in Figure 1. The working gas was air. The experiments were done under atmospheric pressure.

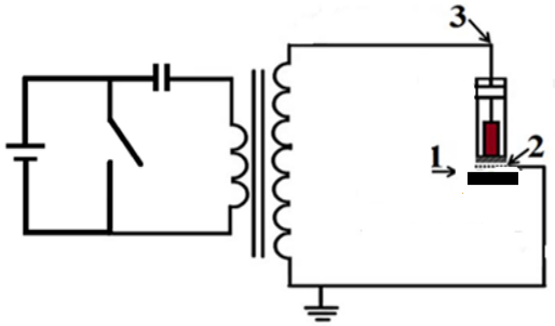

Fig. 1. A schematic view of probe with floating second electrode: 1) bacterium sample; 2) earth electrode; and 3) high voltage electrode.

2.2. Bacteria preparation

The *Pseudomonas aeruginosa* and *Staphylococcus aureus* bacteria were taken from the Department of Biology of the University of Tehran in standard strains. To prepare the solid cultivation medium, the Agar powder (Tryptic soy agar, Merck 1.05458.0500 Caso Agar) was dissolved in pure water. Then, the solution container was put in an autoclave. Next, prior to cooling the solutions, they were poured into plastic dishes. After 2 to 3 minutes, they set like jelly. After this stage, the dishes were put under an anti-microbial hood for 24 hours to ensure that they were totally clean and sterilized. The liquid cultivation medium used in this experiment was *Brot* nutrient. Like the solid medium, the liquid nutrient was dissolved in pure water. The solution was put in an autoclave and sterilized. For bacteria propagation, one colony of bacteria from the solid medium was chosen and put in the liquid medium. The sample from the liquid medium was cultivated in two stages, so the bacteria became stronger. Then, the bacteria sample was placed in the incubator for 24 hours. A small amount of last part bacteria was injected in the liquid cultivation medium. Again, the solution was put in the incubator for 12 to 14 hours to make the sample ready. The bacteria solution was put in a shaker to detach the bacteria colony. The excess liquid was poured out, and the colony was deposited. The opacity of the solution showed the density of the bacteria. The half McFarland method was selected to measure the density. If the opacity of the solution was equal to the opacity of the half McFarland solution, then the density was about $6x10^8$ colony-forming units (CFU). The deposited bacteria were dissolved in the phosphate buffered saline (PBS). At this stage, a small number of bacteria was added step by step to the PBS to reach the opacity of the half McFarland. Thus, the bacteria suspension with a density equal to $6x10^8$ would be reached. For thinner solutions, the bacterial suspension and PBS were mixed with a 10 to 90 ratio, respectively, which resulted in a solution with one lower order of magnitude. For instance, blending 100 microliters of the 10^8 CFU bacteria suspension with 900

microliters PBS, resulted in a solution with a 10^7 CFU density. After preparing the bacteria suspension, 100 microliters with a $6x10^4$ CFU density was placed in each Petri dishes containing the cultivation medium and treated by DBD plasma. Then, the samples were dried by sterilized air for 15 minutes. The samples were exposed to the plasma for a certain time and voltage. Next, they were put in an incubator at 37C for 24 hours. All these stages were done under a microbial hood in sterilized conditions. Figure 2 shows the Petri dishes in different conditions.

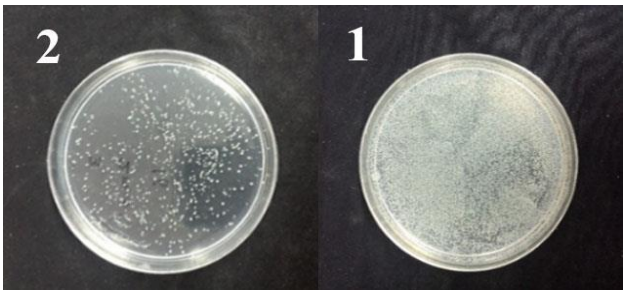

Fig. 2. Petri dishes containing bacteria after cultivation 1) Control sample 2) Under treatment sample

The specified parameters in this study were the time of treatment and the duty cycle of the plasma. Table 1 presents the parameters and their levels.

Table 1 .parameters and their levels

Level	Treatment Time (s)	Duty Cycle
1	30	12
2	60	16
3	90	
4	120	

Pictures of the samples were taken to detect the number of deactivated colonies after plasma treatment, and the deactivation rate was determined by a colony counting machine. The area calculation method was used for densities above 10^7 CFU due to the uniform distribution of bacteria [23].

3. Results and discussion

The number of deactivated *Pseudomonas aeruginosa* colonies is shown in Figures 3a and 3b. As the results show, by increasing the time, the number of deactivated colonies proliferated. Initially, the slope of the deactivation curve was very high. The more time went by, the milder the trend became. It also could be concluded that the duty cycle played a big role in the deactivation of the bacteria. The number of active bacteria is less in the 16 duty cycle in the same time period compared to the 12 duty cycle. These charts are depicted in Figure 4a and 4b. The effect of the plasma treatment on *the Staphylococcus aureus* bacteria followed the same trend as the *Pseudomonas aeruginosa*. Figures 5a and 5b show the relation between the treatment time and deactivation in two different duty cycles. These charts imply that all *the Staphylococcus aureus* bacteria were destroyed in nearly two minutes. The process is shown in Figure 6a and 6b for two duty cycles.

In comparison to the *Pseudomonas aeruginosa,* the DBD plasma treatment had a greater impact on the deactivation of *the Staphylococcus aureus* bacteria. Again, amplification in the plasma duty cycle yielded a better deactivation of the bacteria.

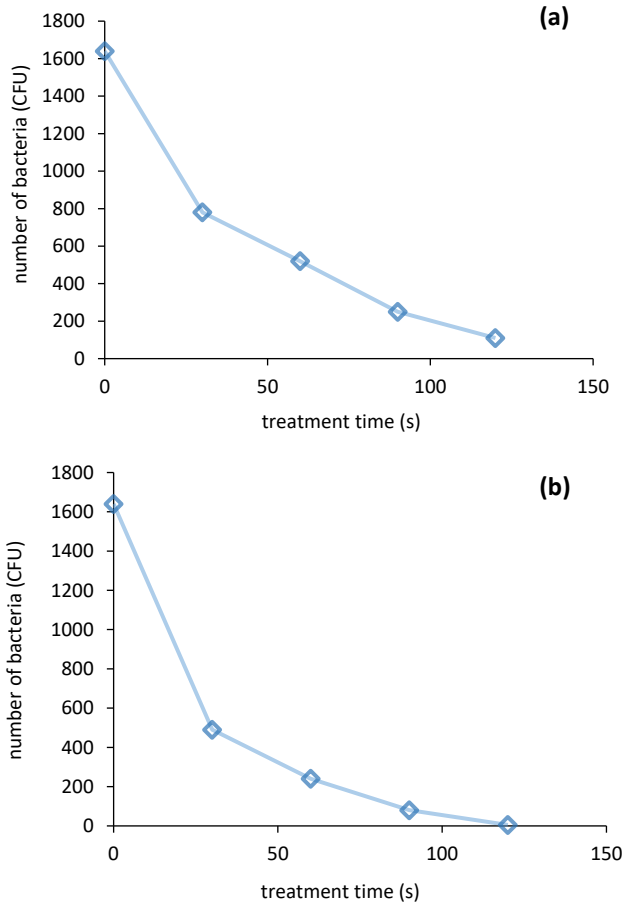

Fig.3. Number of deactivated Pseudomonas aeruginosa colonies in a) 12 and b) 16 duty cycles.

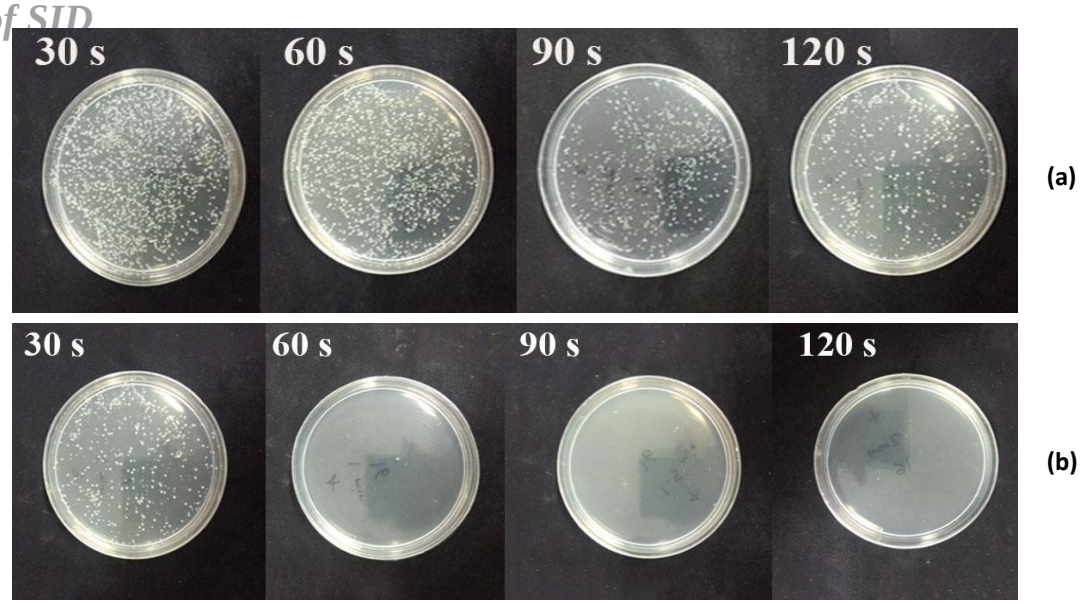

Fig. 4. Deactivation process of Pseudomonas aeruginosa colonies in a) 12 and b) 16 duty cycles**.**

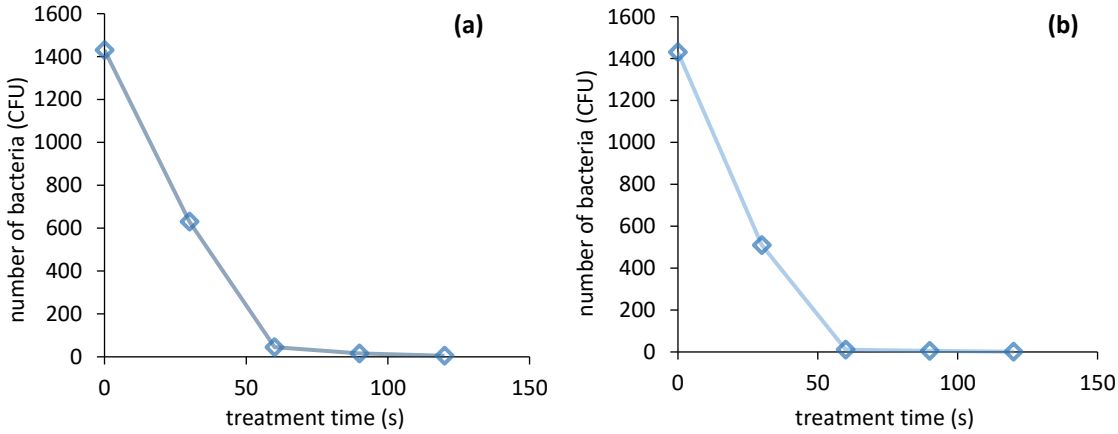

Fig. 5. Number of deactivated Staphylococcus aureus colonies in a) 12 and b) 16 duty cycles.

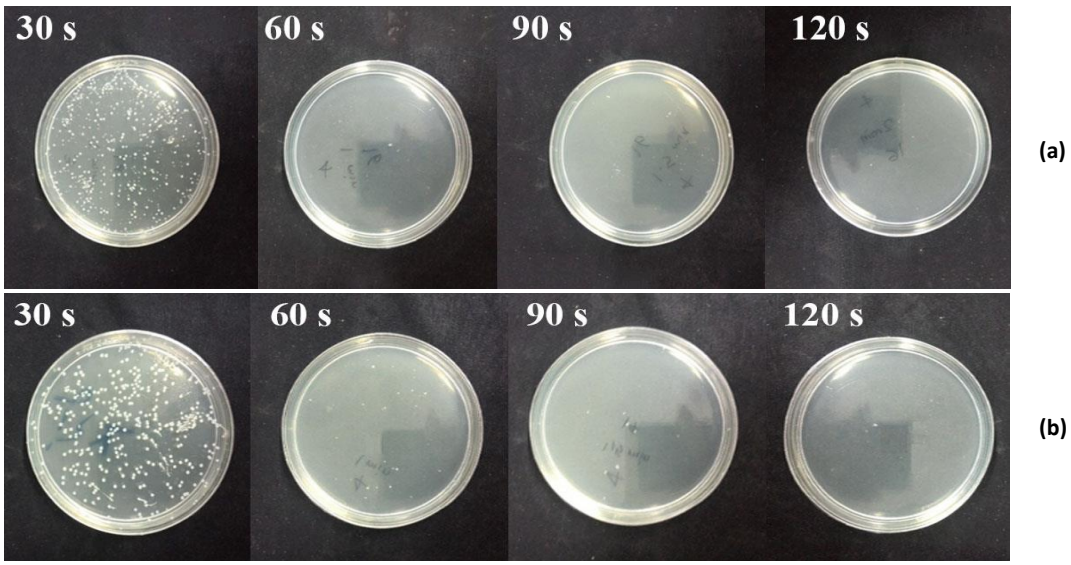

Fig. 6. Deactivation process of Staphylococcus aureus colonies in a) 12 and b) 16 duty cycles.

The innovations of this study included using DBD plasma to treat two bacteria that play big roles in infectious diseases as well as determining the time required to completely deactivate them. In comparison to other studies [16,19,20], which mainly examined the effect of DBD plasma on *Escherichia coli and Bacillus subtilis,* this research focused on two infectious bacteria, namely *Pseudomonas aeruginosa* and *Staphylococcus aureus*. Furthermore, the results implied that 60 seconds was an appropriate time for the complete treatment of the *Staphylococcus aureus* bacteria. The same results have been reported in other studies [24] for the planktonic form of *Staphylococcus aureus,* whereas nearly same results have been obtained [25] for *Staphylococcus epidermidis* using DBD. Therefore, this renders DBD as a good medium for the decontamination of surfaces from infectious bacteria. On the other hand, *Pseudomonas aeruginosa* requires at least two minutes for complete treatment. This time is higher than the times reported in previous studies (Choi et al., 2006).

4. Conclusions

In this study, the effect of DBD plasma treatment on the deactivation of two infectious bacteria has been investigated. The implementation of DBD plasma is an innovative method to destroy bacteria and sterilize hospital waste. Due to the low temperature of this kind of plasma, side impacts like thermal destruction of tissues don't happen in this process. The results suggest that the DBD method is an effective way for the destruction and deactivation of bacteria in a short period of time. The difference between *Pseudomonas aeruginosa* and *Staphylococcus aureus* after treatment comes from their different internal structures. However, both bacteria were deactivated effectively via the plasma treatment.

DBD plasma treatment is a new, quick and environment friendly procedure in which hazardous wastes can be cleaned without the disadvantages of prior methods. Hence, it's a perfect choice for sterilizing medical and hospital wastes.

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