Inactivation of Bacteria using Combined Effects of Magnetic Field, Low Pressure and Ultra Low Frequency Plasma Discharges (ULFP)

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Inactivation of Bacteria using Combined Effects of Magnetic Field, Low Pressure and Ultra Low Frequency Plasma Discharges (ULFP)

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Abstract. Inactivating viable cells at very short application times has been studied using Ultra Low Frequency Plasma (ULFP) at one Kilo Hertz, using an RF source. The targeted fashion is to inactivate *Escherichia coli* (*E. coli*) in the absence and in the presence of magnetic field. Adding oxygen (O_2) to argon (Ar) in the discharge leads to a complete bacterial inactivation, where the inactivation rate increased as the concentration of O_2 increases. Analyses of the experimental data of the initial and final densities of viable cells, using survival curves, showed a dramatic inhibitory effect of plasma discharge to the residual survival of microbial ratio due to the influence of the magnetic field.

1. Introduction

Plasma is currently widely used in different fields due to the wide temperature range enables various applications for plasma technologies [1-4]. These applications include surface etching, biomedical, surface coatings, surface modification, waste destruction, gas treatments, chemical synthesis and machining. Nevertheless, many of these techniques have not been industrialized. Inactivation factors of spore-forming bacteria using low-pressure plasmas and comparative studies of low pressure plasma characteristics between different working gases for sterilization was carried out through different articles [5-6]. So far, depending on the discharge conditions, most of the articles did not take the trend of the influence of the magnetic field on the cold plasma which is one of the oldest problems in plasma physics and remains of great interest in plasma fusion studies. Recently, this has been an important fashion in many plasma discharge used in processing sterilization [7], because the application of a magnetic field results in enhancement of some desirable features of specific plasma sources.

In the present study, it has been suggested to use ultra low frequency plasma (ULFP) radiations for killing some microorganisms adhering to surfaces under the influence of magnetic field. The objective is to investigate the inactivation characteristics of Escherichia coli (E. coli) bacteria, at very short application times, low-pressure and (ULFP), moreover many parameters affecting on the survival curve shapes will be discussed, such as concentration of oxygen to the argon plasma discharge, the presence of the magnetic field, and the direct exposure time.

2. Experimental setup

The CCP plasma system consists of a stainless steel chamber that has few glass ports, connected to vacuum system and evacuated to 10^{-4} torr. The used gases were then admitted to the system via needle valves. As shown in Figure 1, more details about the system, the RF circuit and the plasma cell has been discussed previously in many articles [8-11].
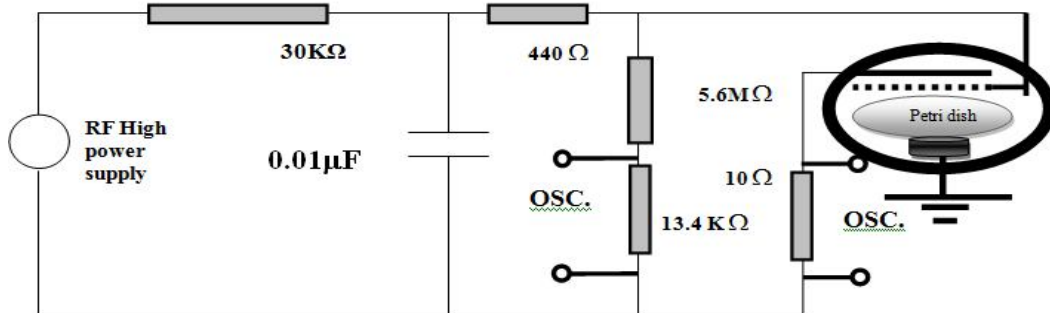

Figure 1. The RF electrical discharge circuit**.**

Biological material can be exposed to plasma in direct exposure as shown in Figure 2, where the treated Petri dishes (containing *E. coli* bacteria) were in direct contact with the plasma. All plasma generated agents, including charged particles, come in contact with the sample (Petri dishes-containing bacteria). The amount of heat transmitted to the sample is increased, the charged particles play a role since they sputtering the sample, and many of the short-lived neutral reactive species also reach the sample. Thus the main inactivation factors by plasma are heat, charged particles, reactive species, and UV radiation.
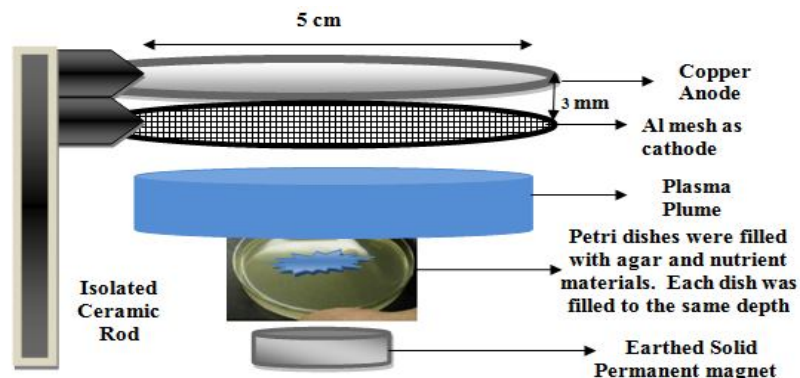

Figure 2. A schematic diagram of the plasma cell.

To show a clear visual effect of the plasma discharge on inactivation process of *E. coli* bacterium, and to demonstrate it in an observable way, a small admixture of oxygen to argon and a small circular solid permanent magnet of few hundreds of gauss pass below the Petri dish (the diameter of the magnet is less than the diameter of the Petri dish) to increase the inactivation efficiency. The tests were conducted as follows: live culture of *E. coli* spread over Petri dishes, two extra Petri dishes were kept as controls. For different times, five Petri dishes were exposed to Argon discharge, five Petri dishes were exposed to Argon- oxygen discharge for 1% O_2, five Petri dishes were exposed to Argon-oxygen discharge for 3% O_2, and finally five Petri dishes were exposed to Argon-oxygen discharge for 3% O_2, in the presence of magnetic field. In all of these tests, the plasma-treated samples were prepared as follows it has been previously suggested [12-14]. The bacterium (*E. coli*) was the organism of choice for our work. An overnight culture containing approximately 10^9 cell-forming unit, cfu/ml was prepared. One of the wells was kept as control while the other wells were exposed to the plasma discharge for two different treatment times. The plasma was in contact with the agar (the

plasma discharge cover the dishes). After exposure, all the Petri dishes were incubated overnight at 37 $^{\circ}$C.

3. Results and discussion

When gas breakdown take place, a sudden increase in the rate of ionization, particularly near the cathode mesh, was observed. Since positive ions move in the electric field much more slowly than the electrons (due to their masses), the electrons were swept rapidly far away from the mesh and a dense positive space charge was formed above the mesh [15]. When an object exposed to plasma, such as a Petri dish contains live culture of *E. coli*, the dish bombarded by both electrons and positive ions, but will initially collect much more electron current due to the higher mobility of the lighter electrons [16]. As a result, the Petri dish becomes negatively charged relative to the plasma bulk. At some negative potential, the electron and ion fluxes will become equal since more electrons are repelled from the Petri dish and positive ions are accelerated toward it to begin the inactivation process**.**

In this study, bacterial inactivation using ULFP, was studied under two the absence and the presence of magnetic field, and in the presence of oxygen.

3.1. Absence of magnetic field

It was noticed that by increasing the exposure time to Petri dishes from 4 min, at the beginning of the inactivation process to 20 min, slightly a complete process. By increasing oxygen ratios to argon from 1% to 3 %, the inactivation region appears as holes of the etched surface of *E- coli* culture and become wider, then contacted together to form more wider holes as shown in Figure (3, groups A, B, and C). The similarity in the surface morphologies implies that the etched surface processes are a self-similar structure in time scale.
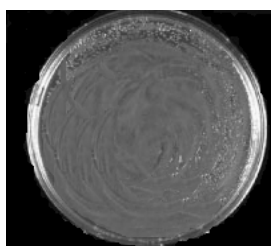
The Control

Exposure Time	Group A Pure Argon	Group B Ar/1% O$_2$	Group C Ar/3% O$_2$
16 min			
20 min			

Figure 3. In the absence of magnetic field, inactivation process of *E. coli* bacteria increased and the exposure time decreased, as the addition values of O_2 increased.

3.2. Presence of magnetic field

When the same parameters for the exposure time and the adding of the oxygen ratio, in the absence of the magnetic field, a dark circles in the center showed no growth regions, as shown in Figures (4) for exposure time from 2 min to 10 min. Around these regions, a dense mat of bacterial growth covers the agar and masks the dark background. We noticed that the effect of plasma discharge on *E. coli* bacteria increased at small exposure time, approximately half the value at time needed for inactivation process in the absence of the magnetic field. From these photographs shown in Figures (4), one can conclude that a centered circular area of the inactivated region increases with increasing the treatment exposure time. Furthermore the area of the inactivated region is much greater when oxygen was added to argon using Ar/3% O_2.
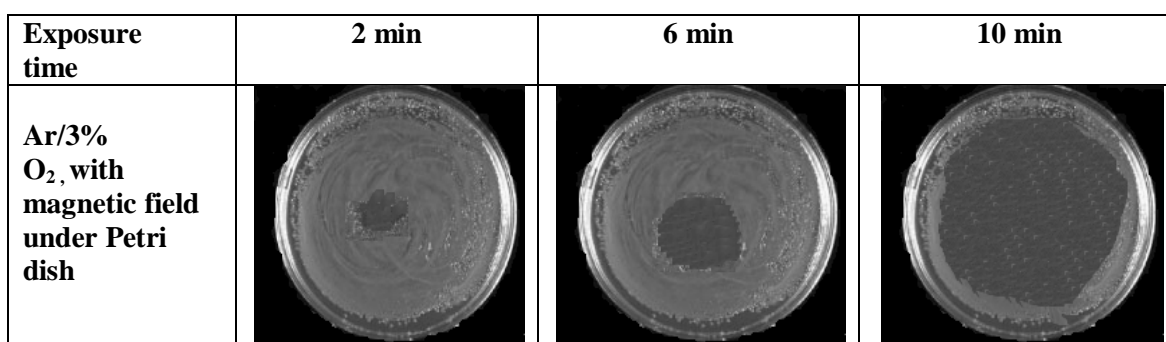

Exposure time	2 min	6 min	10 min
Ar/3% O_2, with magnetic field under Petri dish			

Figure 4. In the presence of magnetic field, inactivation process of *E. coli* bacteria increased and the exposure time decreased as the addition values of O_2 increased.

3.3. Survival curves

In this study the survival curves were focused on the effects of oxygen and magnetic field. Figure (5) showed the survival curves using Ar/0% O_2, Ar/1% O_2 and Ar/3% O_2, in the absence of the magnetic field. For pure argon, there are two phases, phase one gives the fact that radicals from the gaseous phase are involved and given the limited penetration depth of UV photons of plasma discharge. Due to radicals, electrons and positive ions, the destruction of isolated viable cells by UV photons dominates during this phase and phase two which is partly dependent on the erosion rate of the various materials (coats, debris, dead cells) covering still viable cells [17-19].
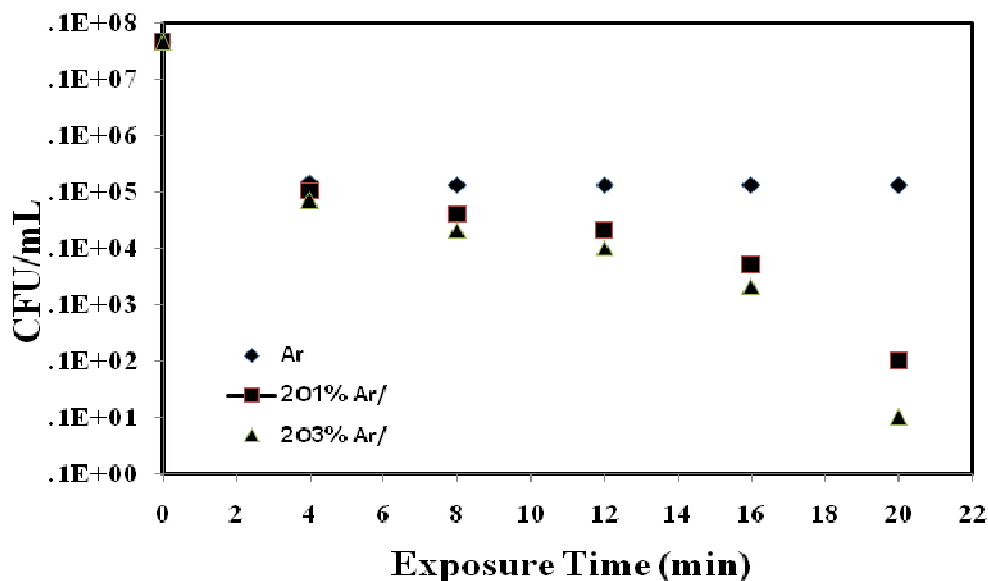
Figure 5. Survival curves of *E. coli* cells exposed to a flowing afterglow, adding O_2 to argon in the discharge without magnetic field.

The survival curve for Ar/1% O_2 and Ar/3% O_2, contains phase three beside the two previous phases with the same characteristics. Phase three starts when the viable cells that were not inactivated during phases 1 and 2 have been sufficiently cleared from debris for the UV photons to finally kill them. This explains why the D_3 time is very often close to D_1 as shown in Table (1) and why this third phase is observed immediately before sterilization is achieved in all cases. Bacterial spore inactivation is ultimately the result of the DNA destruction by UV photons [20-23].

Table 1. D- values changes due to the increasing of the concentration ratio of O_2%

D - Values (min)	D_1	D_2	D_3
Argon	2.1	18	-
Argon/1% O_2	2.1	6	2
Argon/3% O_2 in the absence of magnetic field	2.1	7	2.1
Argon/3% O_2 in the presence of magnetic field	1.2	4.3	1.5

The survival curves for argon/3% O_2 under the influence of magnetic field due to strong permanent magnet contain three phases as in the absence of the magnetic field, but the D values in the presence of magnetic field were less than those in the absence of magnetic field due to the low values of the exposure time as shown in Figure (6).
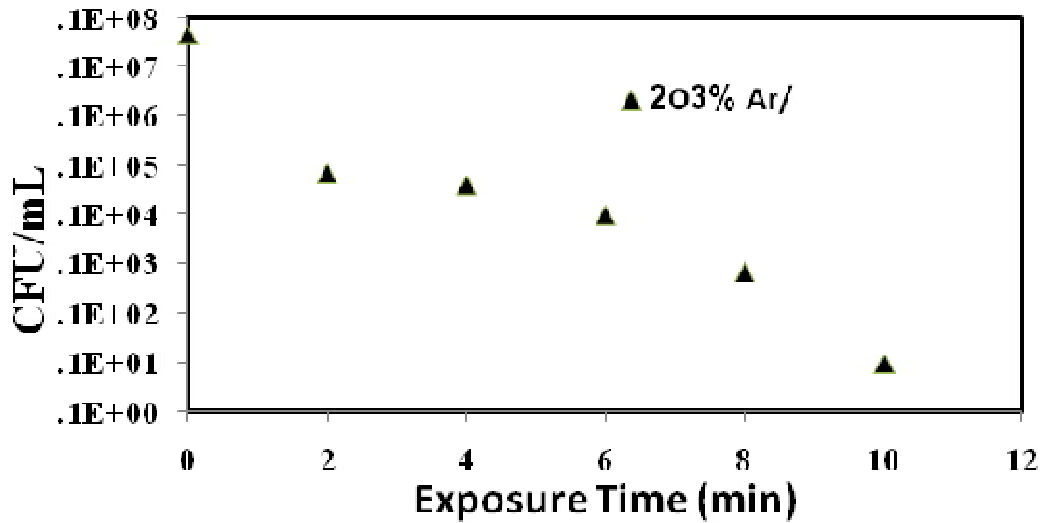
Figure 6. Survival curves of *E. coli* cells exposed to a flowing afterglow, adding 3%O_2 to argon in the discharge with magnetic field.

The longer treatment period and the higher oxygen concentrations increased the ability of low pressure plasmas to inactivate bacteria, as shown in Figure (7). Furthermore, the dramatic inhibitory effect of plasma discharge for the residual survival microbes ratio, in the presence of the magnetic field, was also measured (Figure 8).
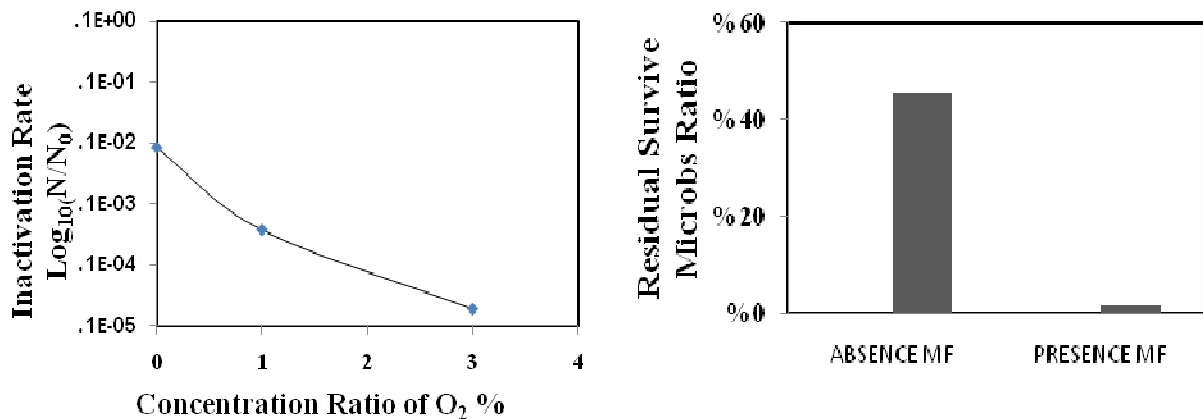

Figure 7. The bacterial inactivation rate increased as the concentration ratio of O_2 % increased.

Figure 8. A dramatic inhibitory for the residual survival of microbe ratio in the presence of magnetic field.

Due to the magnetic field strength B (reach to 100 gauss), the Larmor radius [L_R] decrease, the diffusion coefficient across the field is reduced [L_R], then the electro temperature of electron decreases. Moreover, the density of the plasma increases [24-25] and become more dense over the *E. coli* microbes in the same region of the effected magnetic field. When using the magnetic field, the exposure time decreases approximately half the value comparing to those in the absence of the magnetic field.

4. Conclusions

It is concluded that there are many parameters affecting the survival curve shapes, such as the area of the inactivated region is much greater when oxygen was added to Argon using Ar/3% O_2, especially for the longer treatment time. Furthermore, the higher concentrations of oxygen increase the ability of low pressure plasmas to inactivate bacteria. It was found that he presence of the magnetic field increase the density of plasma over the sample, consequently increase the dramatic inhibitory effect of plasma discharge for the residual survival microbes ratio, followed by increase of the inactivation rate of the *E. coli* microbes. In addition decreasing of the exposure time required for inactivation process; The decrement in the electron temperature with a mixing ratio was mainly due to the large inelastic cross section of the mixed gases, and due to the increasing in the sheath around the grid wires affect the survival curves. It was also concluded that the electron temperature is a strong function of a mixing ratio. The cathode mesh (grid) in CCP (Capacitive Coupled Plasma) controlled the plasma parameters in region over the mesh and investigates plasma parameter variations as a function of a mixing ratio in Ar/O_2 plasma. The wires of the grid have a small distance between each other, was used to give ions with high energy. In the survival curves, phase three starts when the viable cells that were not inactivated during phases 1 and 2 have been sufficiently cleared from debris for the UV photons to finally kill them in the presence of the magnetic filed more than in the absence of the magnetic field. Furthermore sterilization is achieved in all cases, bacterial cells inactivation is ultimately the result of the DNA destruction by UV photons.

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