STUDY THE EFFECT OF NON-THERMAL PLASMA ON LOCAL ISOLATE OF *E. COLI*

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ABSTRACT

In this endeavor, three types of non-thermal dielectric barrier discharge plasma (NTDBDP) systems are designed and built locally in custom configurations and investigate the properties of the produced plasma. The difference between these systems is the shape and configuration of the discharge electrode, which plays an essential role in defining the nature of the generated plasma. The first type is two circular plane copper discs, the second is two concentric circular rings of copper, and the third is two concentric cylindrical tubes. Quartz was used as a plate or tube as dielectric material. The optical emission spectroscopy (OES) method was used to analyze the produced plasma spectrum and calculate the various plasma properties (the temperature of electrons, the density of electrons, the frequency of electrons, the Debye length, and the Debye number) in different conditions of applied voltage for all designs. The generated non-thermal plasma was used to inactivate *E.coli* bacteria at different AC applied voltages (18, 20 and 22 kV) and plasma exposure durations (10, 15, and 20 min). The effect of non-thermal plasma was slight on the bacteria at low applied voltage and exposure time. In contrast, the impact positively of the high voltage and treatment time values on the bacteria. The plasma effect appeared effective at these voltages due to the homogeneously and high intensity of produced plasma and high temperature of plasma electrons. Thus, the effect is more effective on bacteria. The re-cultivation of the treated bacteria demonstrated this, as the spread was very little. All operating scenarios had an electron temperature of around $4.27-5.2$ eV, while the electron density was $(1.035-3.6)\times10^{18}$ cm^{-3}. The results demonstrated the distinct effect of electrode configurations on the properties of the produced plasma due to a change in the electric field's distribution in the discharge region, which allowed it to be used in *E. coli* bacteria inactivation.

Keywords: dielectric barrier discharge, non-thermal plasma, electrode configuration.
*** Part of M.Sc. thesis of the 1st auther.**

دراسة تأثير البلازما غير الحرارية على عزلات محلية من البكتيريا القولونية

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المستخلص

في هذا البحث، تم محليا تصميم وبناء ثلاثة أنواع من انظمة بلازما تفريغ حاجز العزل غير الحرارية في تكوينات خاصة ودراسة خصائص البلازما المنتجة. الفرق بين هذه الأنظمة هو شكل وتكوين قطب التفريغ، والذي يلعب دورًا أساسيًا في تحديد طبيعة البلازما المنتجة. النوع الأول عبارة عن قرصين دائريين مستويين من النحاس، والثاني عبارة عن حلقتين دائريتين متحدتي المركز من النحاس، والثالث عبارة عن أنبوبين أسطوانيين متحدي المركز. تم استخدام الكوارتز كمادة عازلة بشكل لوح مستوي أو أنبوب. تم استخدام طريقة مطيافية الانبعاث الضوئي لتحليل طيف البلازما المنتجة وحساب معلمات البلازما المختلفة (درجة حرارة الإلكترونات، وكثافة الإلكترونات، وتردد الإلكترونات، وطول ديباي، ورقم ديباي) في ظروف مختلفة من الجهد المطبق لجميع التصاميم. تم استخدام البلازما غير الحرارية الناتجة لتعطيل البكتيريا القولونية عند جهود متناوبة مطبقة مختلفة (18 و20 و 22 كيلو فولت) ولفترات تعرض مختلفة للبلازما (10 و15 و20 دقيقة). كان تأثير البلازما غير الحرارية طفيفًا على البكتيريا عند جهد ووقت تعرض منخفض. في المقابل، كان التأثير إيجابيًا على البكتيريا لقيم عالية من الجهد ووقت المعالجة. ظهر تأثير البلازما فعالاً عند جهد مطبق مرتفع يعزى الى تكون بلازما متجانسة وذات كثافة عالية عند الجهود العالية وبالتالي زيادة درجة حرارة اللكترونات البلازما المتولدة. وبالتالي، يكون التأثير أكثر فعالية على البكتيريا. وقد أثبتت إعادة زراعة البكتيريا المعالجة ذلك، حيث كان الانتشار ضئيلًا جدًا. كانت درجة حرارة الإلكترون في جميع سيناريوهات التشغيل حوالي $4.27-5.2$ إلكترون فولت، بينما كانت كثافة الإلكترون $(1.035-3.6)\times10^{18}$ سم$^{-3}$. وأظهرت النتائج التأثير المميز لتكوينات الأقطاب الكهربائية على خصائص البلازما المنتجة بسبب تغير توزيع المجال الكهربائي في منطقة التفريغ، مما سمح باستخدامه في تعطيل بكتيريا الاشرشية القولونية.

الكلمات المفتاحية: تفريغ حاجز العزل، البلازما غير الحرارية، تركيب الاقطاب.
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INTRODUCTION

Recently, compared to low-pressure plasmas, non-thermal atmospheric pressure plasmas have gained popularity as a tool for many applications in the fields of medicine (14, 33) environment (11, 12) and agriculture (7, 8). Furthermore, due to the lower surface damage and shallow surface penetration depth caused by non-thermal plasma, it is preferred for material processing applications and surface modification (3, 29). Non-thermal plasmas are commonly produced by various types of electrical discharges and are described as partially ionized gases containing charged (electrons, ions, radicals) and neutral particles, as well as photons emitted when electrically excited molecules dissociate (20) Plasma contains energetic electrons, ions, active molecules, free roots, intense ultraviolet radiation, and energetic electric fields. All these components contribute to bacteria inactivation and then an effective sterilizing process (19). When the temperature of electrons in the plasma medium is much higher than that of neutral gases and ions, the plasma is called non-thermal plasma (22). Dielectric barrier discharge (DBD) is a widely used discharge system that plays a crucial role in generating non-equilibrium plasma at atmospheric pressure. This system is known for its ability to produce plasma with lower power and cost (6). Alternating current (AC) discharge is generated by applying a periodic electric field between two electrodes, where one (or both) electrodes are covered with a dielectric material such as glass or quartz to suppress large discharge currents (10) The discharge electrodes are an essential component of DBD plasma, so the material, shape and configuration of these electrodes play an indispensable role in the characteristics of the generated plasma (10, 31). Electron temperature and electron density are the main parameters characterizing the resulting plasma; there are many ways to estimate these parameters, some of which are direct, such as the Langmuir probe, which provides spatially accurate measurements, while other probes are Indirect, such as Optical emission spectroscopy (OES) (1) that used in present study. Compared to direct methods, OES is a spectroscopic method that can analyze plasma spectra without causing any interference in the plasma medium (1, 25). Boltzmann plot is a simple and widely used method. It is based on the analysis of optical radiation emitted by the plasma to estimate the electron temperature. The Boltzmann diagram is particularly useful in the case of local thermal equilibrium (LTE) (1, 31). Based on OES spectral analysis T_e, can be calculated using the Boltzmann relation expression (25):

$$\ln\left(\frac{\lambda_{ji}I_{ji}}{hcA_{ji}g_{ji}}\right) = \frac{-1}{kT_e}(E_j) + \ln\left(\frac{N}{U(T)}\right) \qquad (1)$$

where λ_{ji}: is represent the wavelength, I_{ji}: is the relative intensity of the emission line among the energy levels (j and i), h:is Planck's constant, c: is the speed of light, A_{ji}: is the transition probability of spontaneous radiative emission from the level j to the lower level i, g_{ji}: is the statistical weight of the emitting upper level j of the studied transition, E_j: is the energy of excitation, k: is the Boltzmann constant, N: is the total number density of atoms in the ground state and U(T): is the partition function. Another important factor is the electron density, usually measured from the Stark expansion, which defines the plasma environment and creates the equilibrium structure. This can be determined from the line width as follows (23):

$$n_e = \left(\frac{\Delta\lambda}{2\omega_s}\right) N_r \qquad (2)$$

where $\Delta\lambda$: is the line full width at half maximum (FWHM), and ω_s: is the Stark broadening parameter, which can be found in the standard tables, N_r: is the reference electron density equal to 10^{16} cm^{-3} for neutral atoms and 10^{17} cm^{-3} for single charged ions. Most strains of *E. coli* are harmless, but some serotypes can cause severe food poisoning in the host and are occasionally responsible for product recalls to avoid food contamination (32). Harmless species are part of the normal intestinal flora. It may benefit the host by producing vitamin K2 and inhibiting intestinal colonization by bacterial pathogens (27). *E. coli* are rod-shaped, straight, 2.0 μm long and 0.25-1.0 μm in diameter, occur singly or in pairs, motile with peritrichous or sessile flagella, non- self- sporulating, aerobic and facultatively anaerobic, respiratory type and metabolic. Chemoorganotrophs (30). *E. coli*, a common inhabitant of the human

gastrointestinal tract, plays a crucial role in our health. It forms a symbiotic relationship with its human host, often lasting for years, and may benefit the host by producing vitamin K2 and inhibiting intestinal colonization by bacterial pathogens (27). However, these beneficial strains can turn harmful in individuals with weakened immune systems or compromised gastrointestinal defenses, leading to conditions like peritonitis (13). While *E. coli* typically resides in the intestines, it can cause infections when it migrates to other parts of the body where it wouldn't normally be found. This migration can lead to serious health issues, underscoring the potential risks associated with *E. coli* infections (17). The mechanism of bacteria inactivation is due to charged particles such as ions or free electrons participating in the sterilization process. The layers of the cells may selectively absorb some charged particles of different electrical properties in different regions. The electric forces among them can exert pressure or stress, causing distortion, transmogrification, and rupture of the cell walls. Furthermore, the cracks or holes induced by charged particles will facilitate the invasion of free radicals and UV radiation, thus accelerating the sterilization process (15, 18). While previous studies on the use of non-thermal plasma have shown that it is useful and effective in inactivating microorganisms by destroying their membranes and compounds inside the cell, it has been shown to decrease significantly when using high-voltage plasma for 30-180 seconds (28). Many examples witnessed the bacterial effect of non-thermal plasma on bacterial colonies on an agar plate at 1000 watts. The plates were incubated for 24 hours, and after treatment, the surviving groups that formed new colonies were displayed. Research by Mahmuda and others has demonstrated the potent antibacterial effect of non-thermal plasma treatment. Their study aimed to sterilize a therapeutic device using plasma at an optimal temperature and pressure for bacterial inactivation. The results were compelling, with the treated groups showing a significant inhibition of bacterial growth, reducing it by approximately 99% compared to the untreated bacteria (19). The present study aims to build a non-thermal plasma system to treat *E. coli* bacteria (solid and liquid) through the effect of non-thermal plasma properties generated using different types of discharge electrodes.

MATERIALS AND METHODS

Source of *E.*coli

The bacteria were taken from the Department of Biology - College of Sciences - Mustansiriyah University - Republic of Iraq.

Activation and preparation the bacterial isolate

The culture of bacterial *E. coli* was activation by mixing 13 grams of nutrient broth (Oxoid nutrient agar CM0003, United Kingdom), in a liter of distilled water then melted in an autoclave at 121 ^{o}C and 1.5 bar pressure for 15 minutes, then cooled and poured in a sterile plane tubes. After they hardened, the bacteria were cultured using sterile loop, thereafter incubated these tubes at 37^{o}C for 24 hr. Then, they were grown on the solid nutrient agar and kept at 37^{o}C degrees for 24 hours.

Serial dilutions of bacterial isolate were done using normal saline solution and the dilution was compared with the microbe. A spectrophotometer is used to measure the bacterial growth of liquid bacteria (21).

Preparation the indigenously non-thermal DBD plasma system and their effect on *E. coli*

Fig.1 indicates the fabricated indigenously non-thermal DBD plasma system at atmospheric pressure, which consists of two copper electrodes separated by an air gap. A glass plate covers one of these electrodes as a dielectric to prevent sparks between them. The two electrodes for each design were connected to an AC high-voltage power supply (fabricated indigenously), up to 30 V, and frequency up to 35 kHz. The (HR4000CG-UV-NIR, Ocean Optics) optical emission spectrometer was used to analyze the plasma spectrum emitted in the discharged gap between the electrodes.

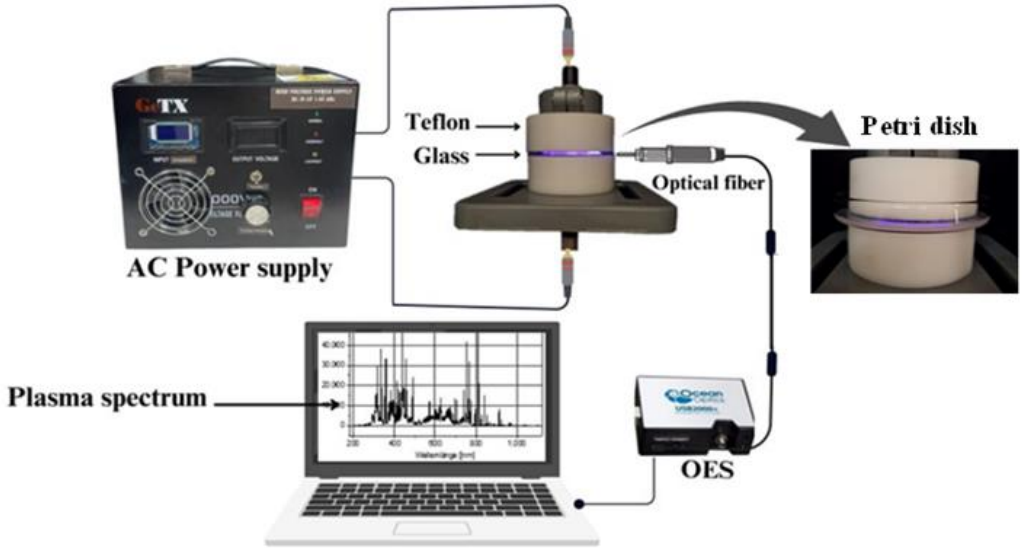

Figure 1. Schematic of used system setup

Three electrode designs were used in this study. The two first designs as shown in Fig. 2 consist of two circular plane copper discs, each surrounded by 1cm Teflon. The second consists of two concentric circular rings of copper. These rings are connected in mid, and the Teflon surrounds each one of the rings. The dimensions of these electrodes are indicated in the figure. Quartz circular plates were used as dielectric material and covered the lower electrode for each design. Non-thermal plasma produced in these designs was used to treat *E. coli* bacteria in solid media by putting a petri dish of bacteria in the gap between electrodes which was 4 mm.

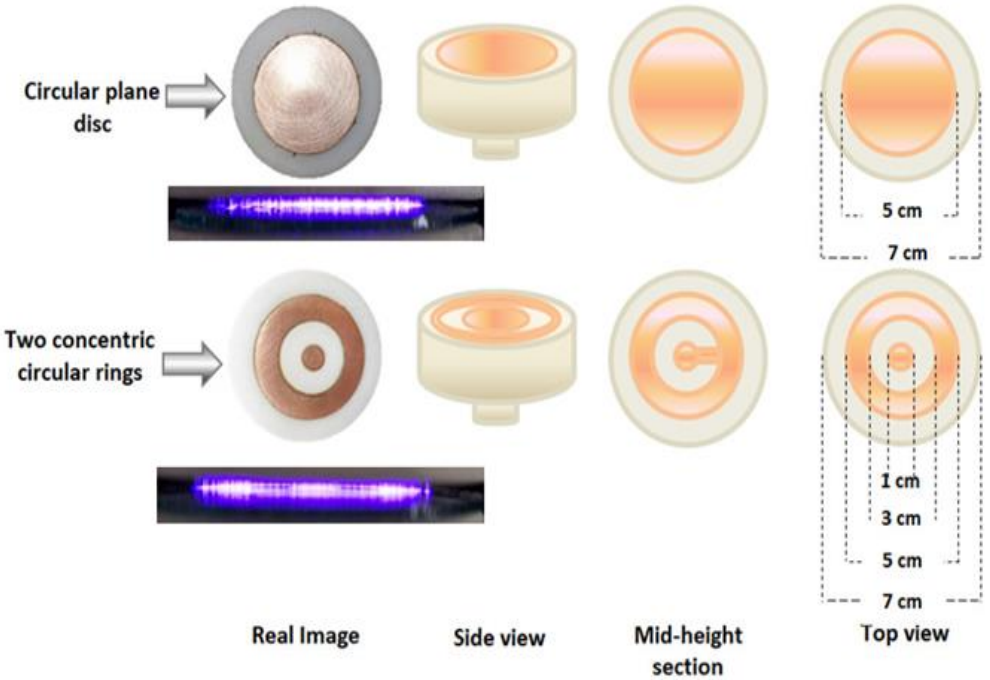

Figure 2. Schematic of the copper electrodes; circular plane discs, and concentric circular rings

Electrodes in the third design, shown in Fig.3, are two concentric cylindrical copper tubes. The inner electrode is a copper cylindrical rod put in a quartz tube, both copper rod and quartz tube put in another quartz tube, and the outer copper electrode surrounds the outer quartz tube. The non-thermal plasma produced in this design was used to treat the bacterial *E. coli* in liquid media by putting the bacteria liquid in the gap between the two quartz tubes, the plasma generation region.

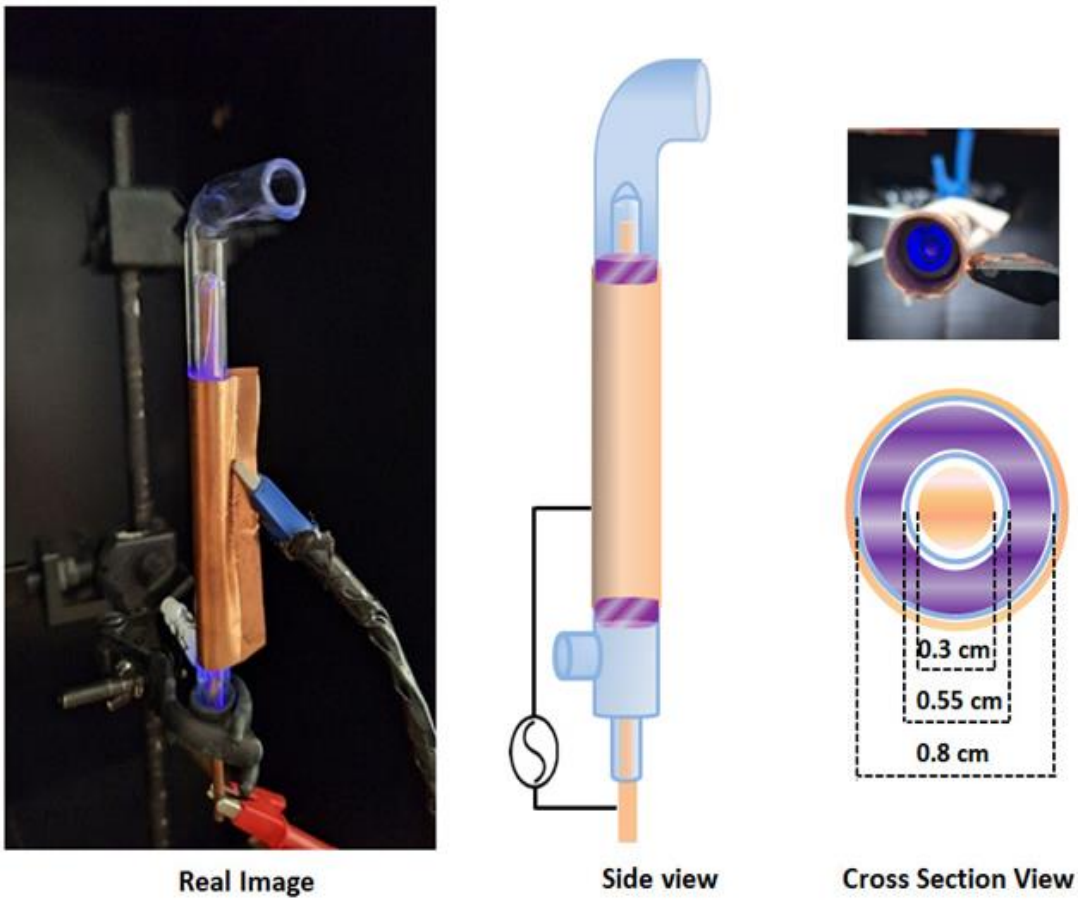

Figure 3. Non-thermal plasma system; image and schematic of the electrode (concentric cylindrical tubes)

RESULTS AND DISCUSSION

Figures 4, 5, and 6 show the emission spectrum of Dielectric barrier discharge plasma produced at different applied voltages. For the first two types of electrodes, the distance between electrodes was fixed at 4mm. For all designs, the results of optical emission spectroscopy for the range of 200 - 800 nm wavelength; according to the NIST database, all measured spectral intensity lines belong to nitrogen ions of NIII and NV (28). For all designs, the intensity of spectral lines increased by increasing the applied voltage due to the increased electric field in the discharge gap. The rising electric field led to more collisions; consequently, it increased the ionization of the nitrogen molecules in the surrounding region, leading to a higher density of charged particles and higher plasma spectrum intensity (1, 10). The same figures show that the intensity of DBD plasma produced between the two circular plane discs was higher than that of two concentric circular rings due to the increase in the area of the discharge region in the first case compared to the second, consequently raising the electric field in the discharge region, then intensity. In the third design, the intensity of optical emission spectroscopy is slight due to the region of plasma production, which makes measuring the spectrum difficult.

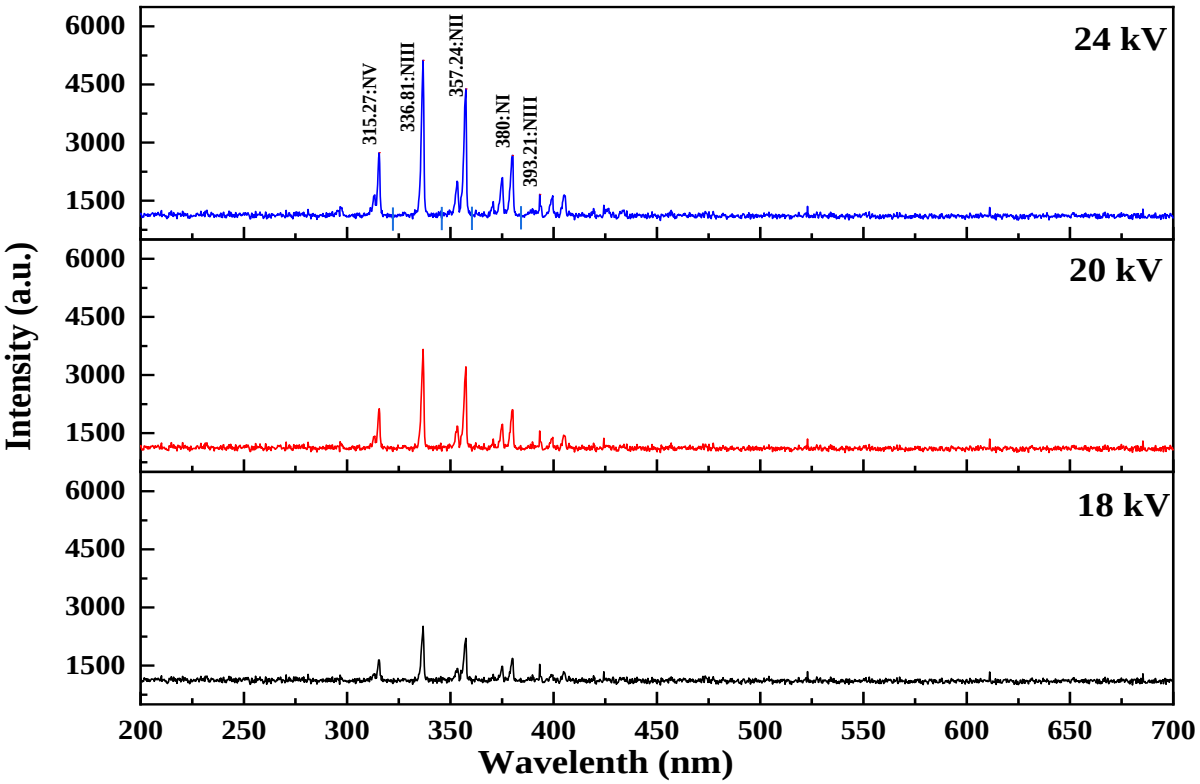

Figure 4. Spectrum of DBD plasma at different applied voltage (circular plane discs)

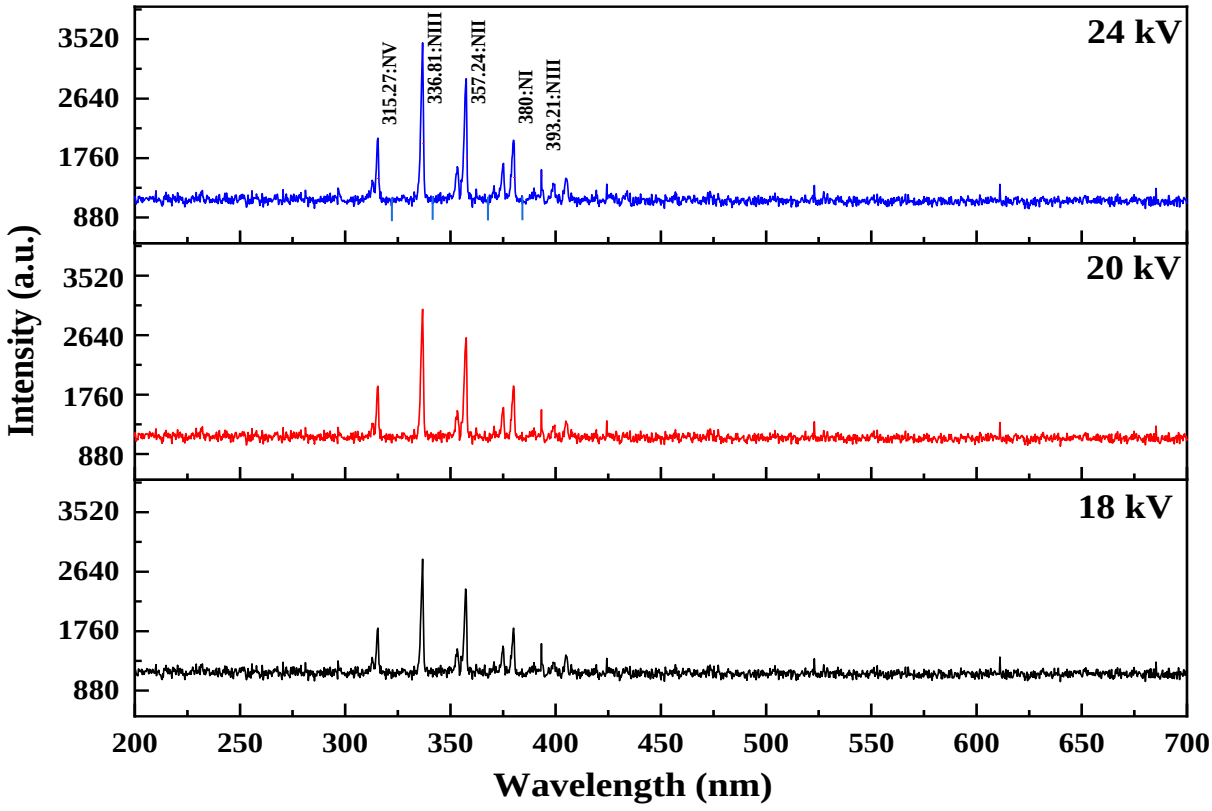

Figure 5. Spectrum of DBD plasma at different applied voltage (concentric circular rings)

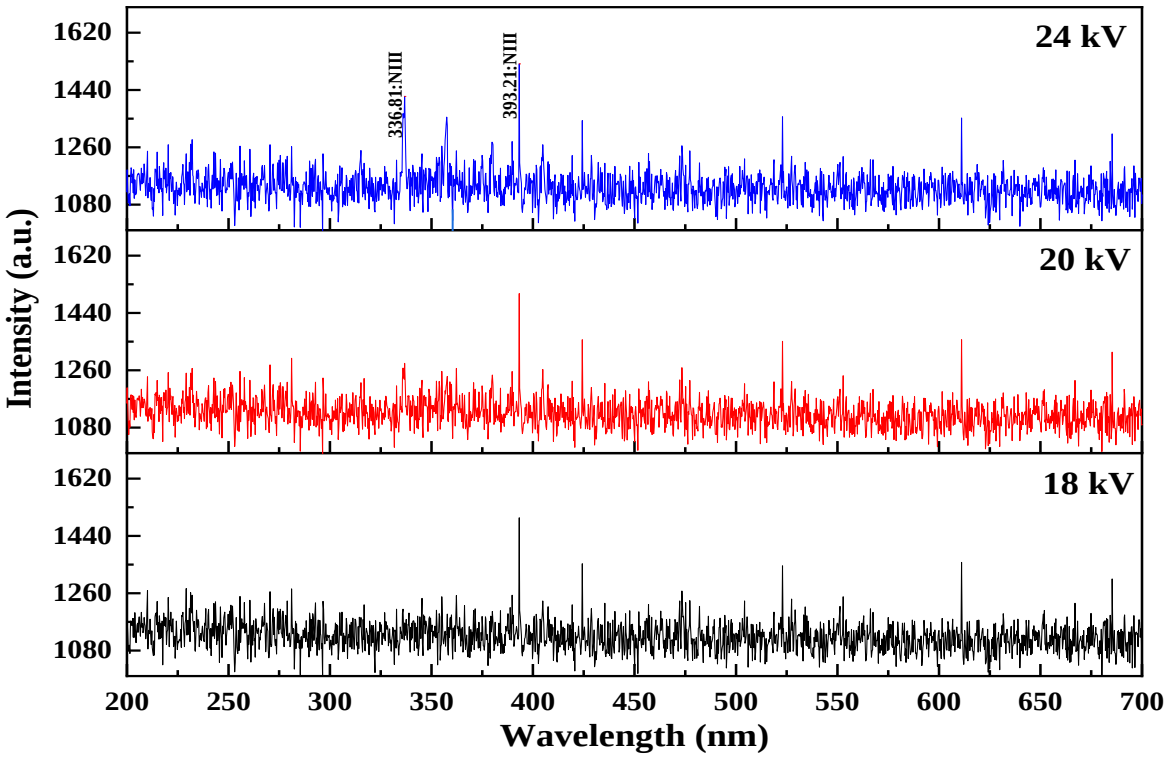

Figure 6. Spectrum of DBD plasma at different applied voltage (concentric cylindrical tubes)

Figures 7, 8, and 9 illustrate the results of calculating the temperature of the plasma electron, which represents the other characteristics of the plasma, including the relative population of energy levels and the speed distribution of particles, according to the Boltzmann plot, that equal inverse slope of the relationship between $\ln(\lambda_{ji}I_{ji}/hcA_{ji}g_{ji})$ and the upper energy level E_j at different conditions of applied voltage and discharge gap distance for all designs. The findings unveiled a significant correlation between plasma intensity and the corresponding electron temperature. As the intensity of plasma spectrum lines escalates, the electron temperature increases. This is attributed to the acceleration of the electrons following exposure to a high electric field, which augments their kinetic energy and, consequently, their temperature. It's important to note that the increase in electric field is directly proportional to the applied voltage. The arrangement and configuration of the electrodes also affect the plasma spectrum intensity, so the plasma spectrum changes for each design at the same applied voltage. Table 1 lists all calculated electron temperatures and densities of the produced plasma at different applied voltage conditions for all designs. The plasma electron temperature for the design of the two circular plane disc electrodes shows a higher temperature than that of concentric circular ring electrodes due to the high electric field generated in the gap between electrodes as explained previously.

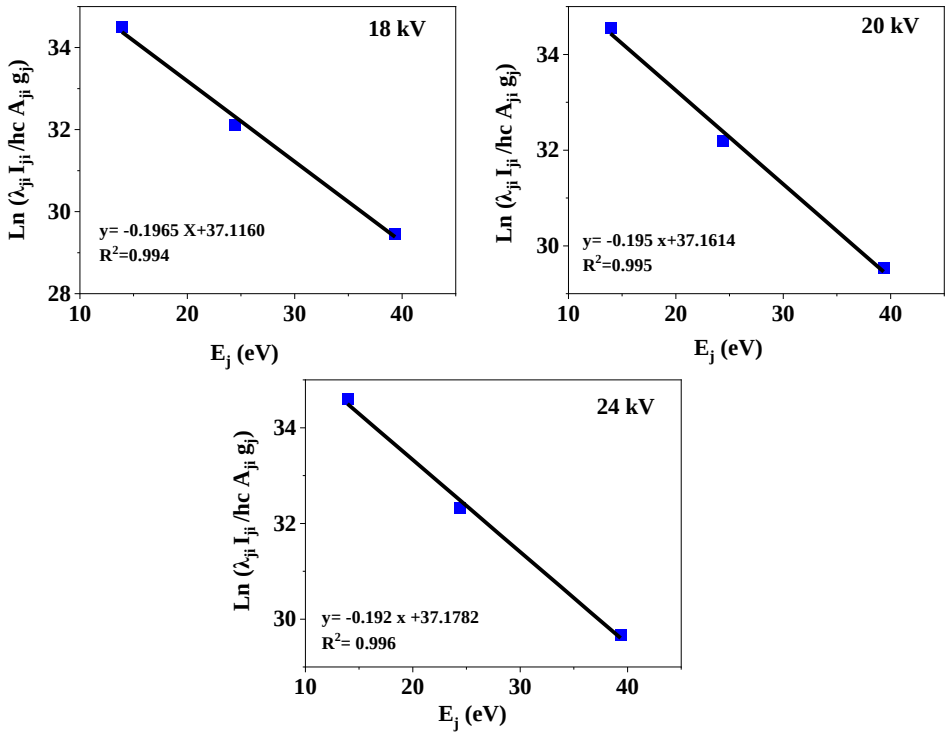

Figure 7. Boltzmann diagram at different voltages (circular plane discs)

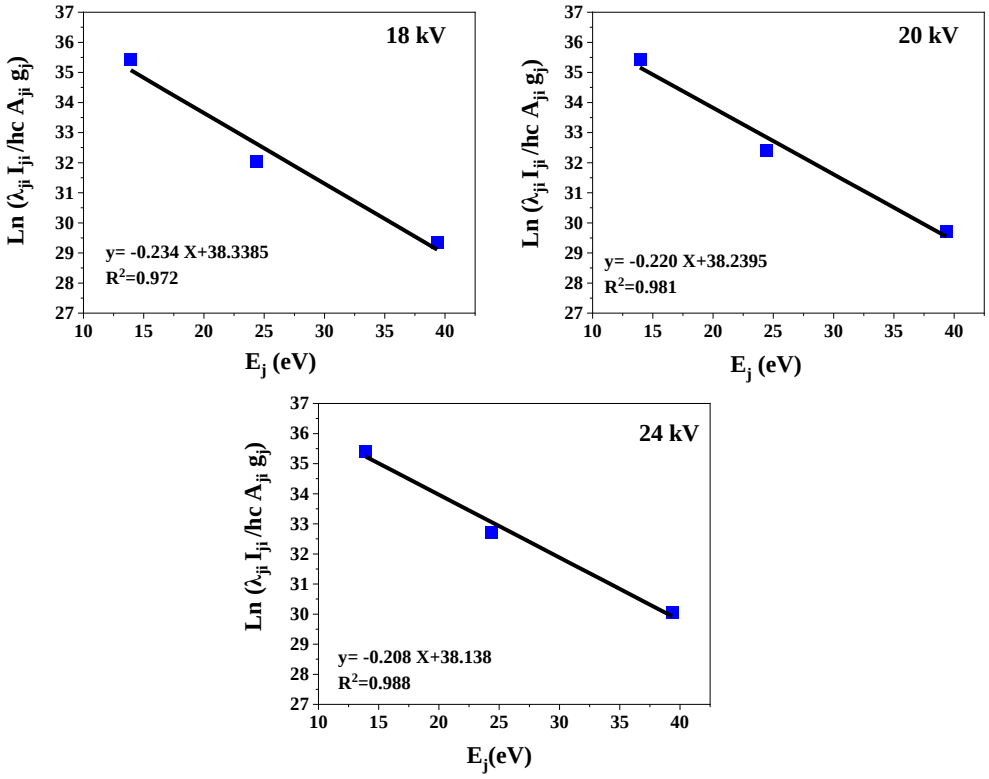

Figure 8. Boltzmann diagram at different voltages (concentric circular rings)

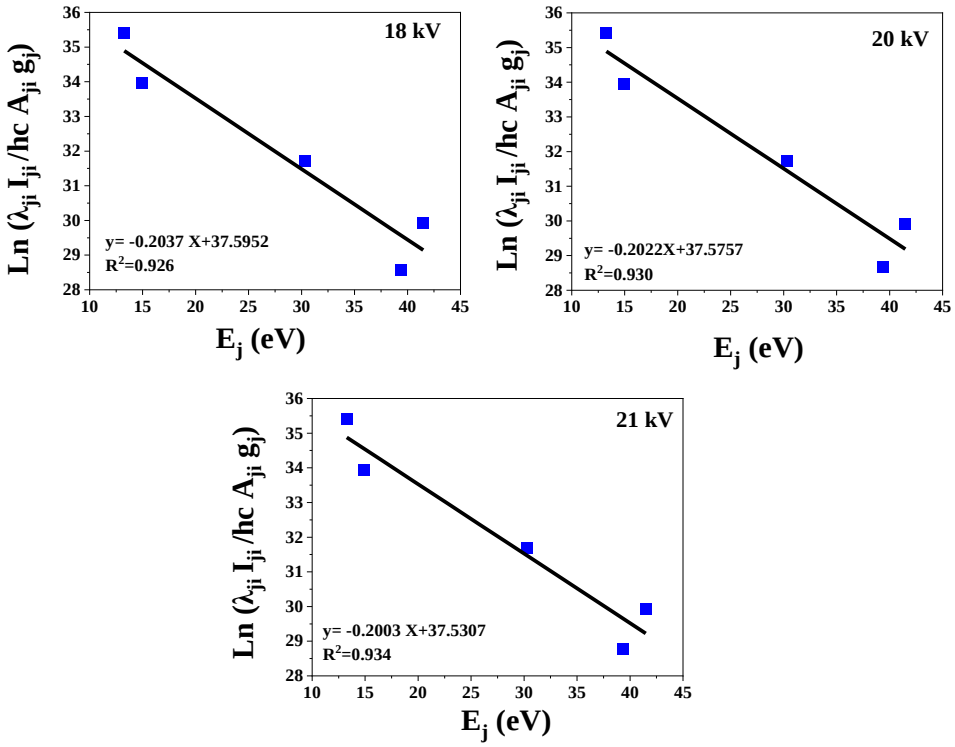

Figure 9. Boltzmann diagram at different voltages (concentric cylindrical tubes)

Table 1. Plasma parameters at different voltages for all design

Applied Voltage (kV)	Circular plane discs		Concentric circular rings		Concentric cylindrical tubes	
	T_e (eV)	$n_e \times 10^{17}$ (cm^{-3})	T_e (eV)	$n_e \times 10^{18}$ (cm^{-3})	T_e (eV)	$n_e \times 10^{18}$ (cm^{-3})
18	5.08	2.62	4.27	1.035	4.90	2.85
20	5.12	2.85	4.54	1.057	4.94	3.3
22	5.20	3.15	4.80	1.087	4.99	3.6

The effect of plasma on E-coli bacteria

High applied voltage produces homogeneous plasma with high intensity due to an increasing ionization ratio, which means more charged particles (ions), free electrons, free radicals, intermediate reactive atoms, molecules, and UV photons resulting from the gas's ionized state. As the voltage increases, the collisions between the electrons and ions between the two electrodes increase; thus, the temperature of the plasma electron will rise. And its effect on the bacteria will be apparent. The plasma designs used in this study were employed to inhibit *E. coli* bacteria in solid and liquid media. The first two designs were used to inhibit bacteria in solid media, while the third design was used for the case of bacteria in liquid media. The selection was made to suit each design to contain the bacteria. Bacteria were exposed to generated plasma under certain conditions, and how the bacteria were affected by plasma was studied. To identify the effect of plasma on inactivation bacteria processes, bacteria were cultivated in a predetermined culture media under laboratory conditions, wherein bacteria multiply themselves, to determine their abundance in culture media; then, the effect of plasma in bacteria inactivation processes, the experiment was repeated twice for all electrode designs to confirm the study's results. Fig. 10 shows the effect of non-thermal plasma generated using the double circular plane copper disc electrode design on *E. coli* bacteria by direct plasma exposure. The petri dish of *E. coli* bacteria in solid media was placed in the gap between the electrodes at 22 kV applied voltage for different exposure durations (10, 15, 20 min). The plasma effect is apparent (region indicated by a yellow dashed line), and the effect region increases with exposure duration. Also, the re-cultivation of bacteria identified the success of the *E. coli* inhibition process.

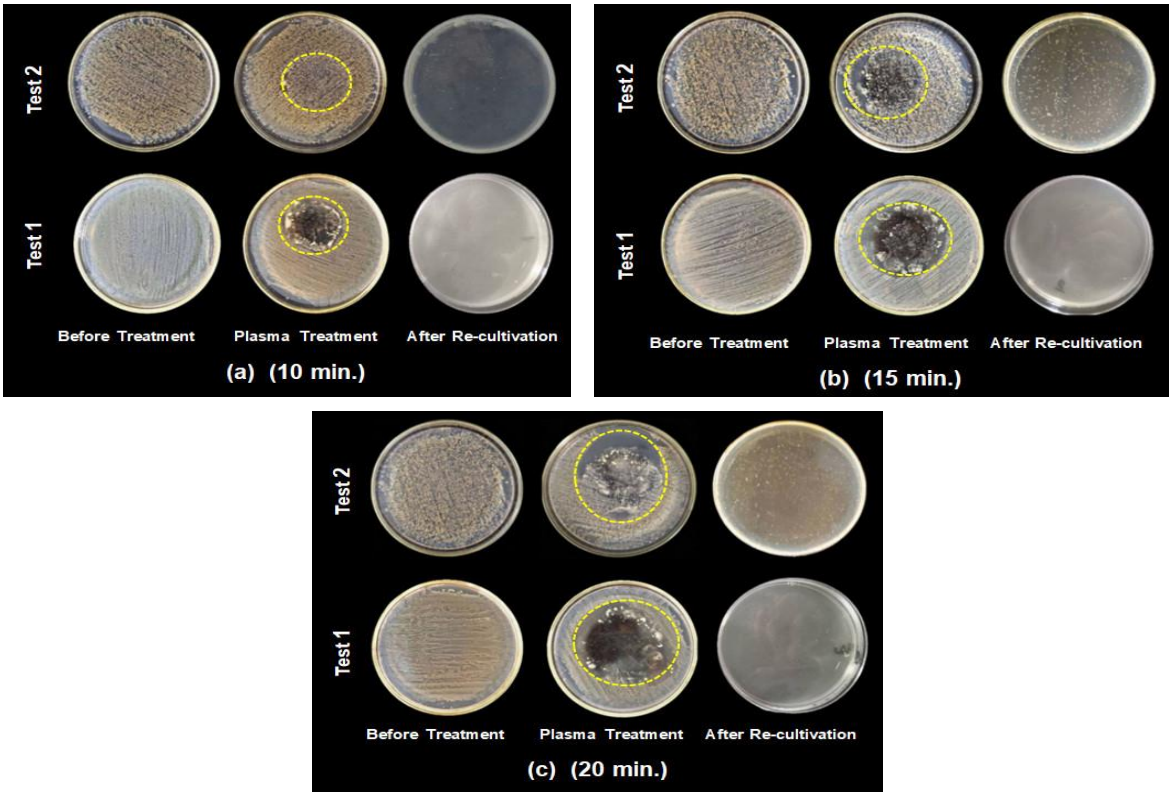

Figure 10. Solid *E.coli* inactivation using non-thermal plasma (double circular plane copper disc electrodes) at 22 kV for different exposure durations

The plasma effect is apparent (region indicated by a yellow dashed line), and the impact of plasma generated on bacteria depends on electrode shape; the effect region on bacteria took a two concentric circular ring as the used electrode shape. The effect region increases with exposure duration. Also, the re-cultivation of bacteria identified the success of the *E. coli* inhibition process.

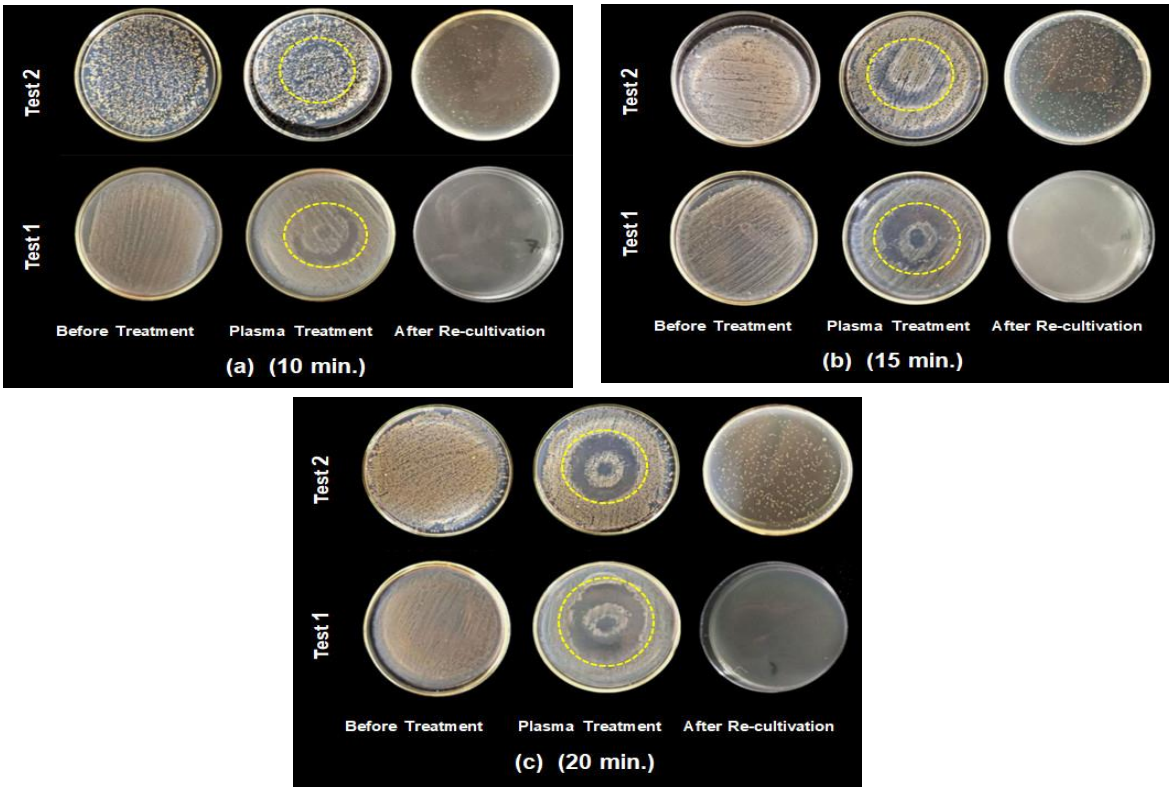

Figure 11. Solid E-coli inactivation using non-thermal plasma (double concentric copper circular rings electrodes) at 22 kV for different exposure durations

The effect of plasma on bacteria in liquid media: The liquid *E.coli* bacteria were treated with the same previous conditions, and positive results were obtained by knowing the bacterial growth reading for each of these conditions and comparing it with the bacterial stock before treatment. Fig. 12 and Table 2 show the effect of non-thermal plasma on the prepared liquid bacteria for different bacterial stock (1.495 and 0.440 cells/mil) at 20 kV applied voltage for different exposure durations (2, 4, and 6 minutes). The viability of the bacteria decreased as exposure duration increased. Fig. 13 and Table 2 show the effect of non-thermal plasma on the prepared liquid bacteria for different bacterial stock (1.495 and 0.440 cells/mil) at 6 min. exposure durations for different applied voltages (18, 20, and 22 kV). The viability of the bacteria decreased as exposure duration increased.

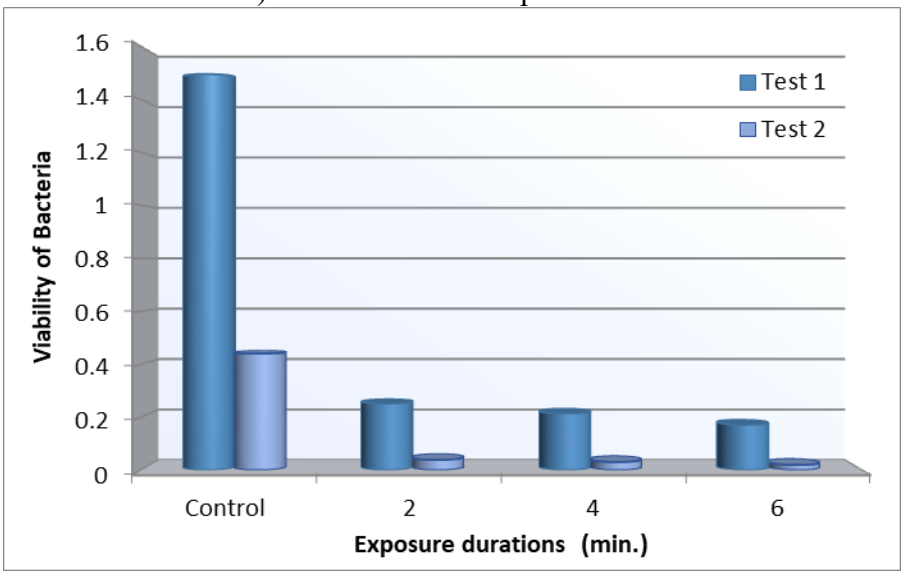

Figure 12. Liquid E-coli inactivation using non-thermal plasma (concentric cylindrical tubes electrodes) for different exposure durations at 20 kV applied voltage

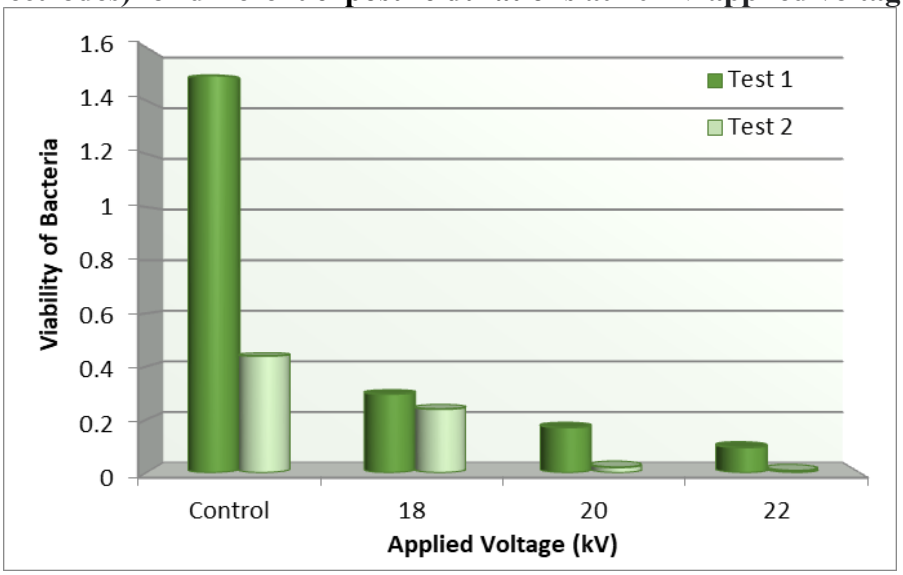

Figure 13. Liquid E-coli inactivation using non-thermal plasma (concentric cylindrical tubes electrodes) for different applied voltage at 6 min. exposure durations

Table 2.The effect of plasma exposure durations on the growth of *E.coli* bacteria liquid at 20 kV applied voltage

exposure durations (min.)	Test 1 Stock (cell/mil)	bacterial growth after treatment (cell/mil)	Test 2 Stock (cell/mil)	bacterial growth after treatment (cell/mil)
2	1.495	0.252	0.440	0.039
4	1.495	0.215	0.440	0.031
6	1.495	0.172	0.440	0.020

Table 3.The effect of applied voltage on the growth of *E.coli* bacteria liquid at 6 min exposure duration

Applied Voltage (kV)	Test 1 Stock (cell/mil)	bacterial growth after treatment (cell/mil)	Test 2 Stock (cell/mil)	bacterial growth after treatment (cell/mil)
18	1.495	0.297	0.440	0.240
20	1.495	0.171	0.440	0.021
22	1.495	0.096	0.440	0.008

Conclusions

The design of the electrodes has an apparent effect on the properties of the produced plasma; this effect appears as a change in the distribution and homogeneity of plasma intensity in the discharge gap between the discharge electrodes as a result of the formation of an electric field with specific properties in this region based on electrodes configuration. Consequently, electron temperature plasma density and all plasma parameters will be sensitive to changes in electrode design. These changes in the plasma properties positively affected the process of significantly reducing bacterial growth, especially at a high applied voltage. The success of the produced non-thermal plasma in inhibiting e-coli in liquid media will allow the employ of this technique in the Sterilization of bacterial-polluted liquids such as water.

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