

## Inactivation of *Escherichia coli* in Water Using an Atmospheric Pressure Plasma Jet

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### Abstract

The aim of this study is to inhibit the activity of efficient of *Escherichia coli* suspended in water using helium plasma jets operating at excitation frequency of 12 kHz of atmospheric pressure. These bacteria are widespread in the environment, especially in drinking water, and their pathogenic effects are extremely harmful. 50 mL drinking water contaminated with *E. coli* was exposed to plasma plume, the treatment time ranged as (5, 10, 15, 20 to 25) minutes. Reactive chemical species such as excited state molecules, ionic compounds and radicals produced in plasma with water detected using the method of photoemission spectroscopy. The bacterial count was reduced from  $235 \times 10^{10}$  CFU/mL to  $42 \times 10^8$  CFU/mL within 5 minutes. The number of bacteria observed a continuous decrease in every five minutes treatments until the number of bacteria per minute reaches  $4 \times 10^2$  CFU/mL after 25 minute. Moreover, the reduction in turbidity of the water sample containing *E. coli* bacteria is clear, as illustrated in two samples. Sample 1 goes from 1.87 NTU control to 0.09 NTU when the period of treatment is 25 minutes, and sample 2 goes from 2.70 NTU control to 0.9 NTU in the same treatment period. An SRE-APHPJ is a promising technique for sterilizing bacteria (*E. coli*) suspended in water.

**Keywords:** non-thermal atmospheric pressure plasma jet, plasma-liquid interactions, reactive species, *Escherichia coli*.

## Introduction

**E**scherichia coli significantly affects human safety (Pathogenic *Escherichia coli*, mainly Shiga-toxin-producing strains (STEC), constitutes a major threat to human safety through fecal-oral transmission via contaminated food/water or direct contact. The clinical spectrum ranges from acute hemorrhagic colitis to life-threatening hemolytic uremic syndrome (HUS), in the first place mediated by Shiga toxin-induced vascular endothelial damage. Therapeutic management is increasingly convoluted by the emergence of antimicrobial resistance (AMR), heightening dangers of therapeutic failure. Moreover, its propensity for foodborne epidemic transmission underscores an ongoing critical challenge to public health system). In water, some of its varieties proliferate quickly. The use of atmospheric pressure plasma jets (APPJs) is among the newest and safest methods in biomedicine. Plasma produces electrons, ions, molecular radicals, and reactive species, all of which have a major effect on the chemical and biological characteristics of many substances. The plasma generated by these methods releases strong UV radiation, shock waves, and active chemical radicals such hydroxyl (OH), atomic oxygen, and reactive oxygen species. These agents are effective for decontamination because of their short lifespans [1]. The plasma jet interacts with the liquid in a variety of ways depending on whether the plasma column is outside of it or whether its tip touches the liquid's surface. Scholarly interest in the atmospheric plasma jet method of inactivating *E. coli* has grown significantly [2–3]. A Plasma-Activated Water (PAW) (denotes to aqueous media treated with cold atmospheric-pressure plasma (CAP), resulting in the generation of reactive oxygen and nitrogen species (RONs). These include, but are not limited to Hydrogen peroxide ( $H_2O_2$ ), Hydroxyl radicals ( $\cdot OH$ ), Nitrate ions ( $NO_3^-$ ), Nitrite ions ( $NO_2^-$ )). Aerosol-producing device's ability to reduce *E. coli* dried onto metal discs investigated in a study. The PAW aerosol sprayed on these metal discs from a distance of 50 or 70 cm for 15 seconds or 1 minute while they were in a  $1m^3$  container [4-5]. Plasma with non-thermal atmospheric pressure used as a method to inactivate germs. A self-designed non-thermal atmospheric pressure plasma jet device used to inactivate the microorganisms. *Escherichia coli* served as the objective microbe in this experiment. After 20 seconds of non-thermal plasma treatment, the *E. coli* bacteria rendered inactive [5-6]. An alternate non-heating sterilizing technique that kills or inhibits microbial development and shields potatoes from PTM infestation is the dielectric barrier discharge plasma device (DBD). According to the exposure duration, a significant negative impact on the exposed

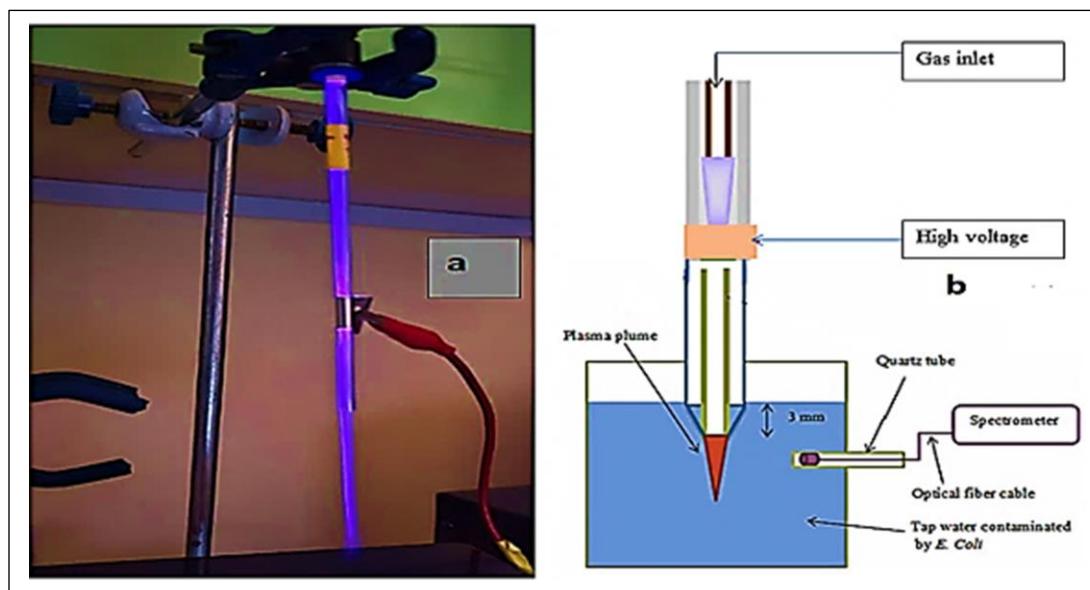
species was noted [8–10]. The internal liquid systems generated a plasma-activated water (PAW) that had a high concentration of nitrogen species. Reduced pH and increased nitrite and hydrogen peroxide levels produced antibacterial effects that were verified against the gram-negative bacterium *E.coli* [11]. The use of atmospheric pressure plasma jets (APPJ) in the food and agricultural sectors, particularly for food preservation and sterilizing applications, has garnered a lot of attention lately [12–13]. The efficiency of reactive species produced in cold plasma treated water (CPTW) for oxidative stress-induced microbial inactivation was examined in a study [14]. The need for sanitizing chemicals can be decreased by using plasma-activated water for cleaning and sanitizing food in the early stages of processing. This benefits aquatic and terrestrial life and lessens harmful wastewater [15]. The purpose of a review was to assess how well cold plasma treatment works to lower various pathogens and spoilage germs in different foods. Furthermore, the impact of plasma variables on the rate of microorganism reduction was evaluated [8, 16]. Non-thermal plasma systems that used plasma interaction above, inside liquid, or in a remote bubbling regime were able to create plasma-activated water [12]. Inactivating the *E. coli* bacteria that is floating in water is the aim of this project. *Escherichia coli*, which is frequently found in soil, food, and the environment. *E. coli* significantly affects human safety. In water, certain varieties of it proliferate quickly. The application of atmospheric pressure plasma jets (APPJs) in biomedicine is one of the safe and contemporary methods. AC power supply can be employed to operate the single-ring electrode (SRE) jets used in this work. A high-voltage electrode connected to the power source and a dielectric tube made comprised the SRE jet. The device components are a power supply of high voltage (0-20) kV alternating current generator, Pyrex tubes of wall thicknesses of 1 mm, a single ring electrode that is made of aluminum with a thickness of 1 mm placed around the Pyrex tube, and the working gas for electrical discharge is helium (He).

**Aim of the Study:** To develop a novel single-ring electrode plasma jet for microbial sterilization of contaminated water, this work investigates non-thermal atmospheric pressure plasma (NTAPP) technology through design and construct an optimized dielectric barrier discharge (DBD) plasma jet by implement a single-ring electrode configuration to eliminate inter-electrode sparking. Assess sterilization efficacy Inactivation kinetics for: Gram-negative (*E. coli* bacteria) and Application focus: water decontamination efficiency (log-reduction CFU/mL).

## Materials and Methods

### Experimental part

An AC power supply can run the single-ring electrode (SRE) system employed in this work, as illustrated in Figure 1. A high-voltage electrode connected to the power source and a dielectric tube comprised the SRE jet. The device's components include a high-voltage (0–20 kV) alternating current generator power supply, Pyrex tubes with walls that are 3 mm thick, a single ring electrode made of 1 mm thick aluminum that is positioned around the Pyrex tube, and helium (He) as the working gas for electrical discharge. The flow meter used to control the flow of helium gas via the flexible plastic tube and secured to the Pyrex tube with a strong lock the procedure takes place by the activation of bacteria in culture dishes prepared for usage, contaminating water using them, and treating it in a locally equipped plasma system.



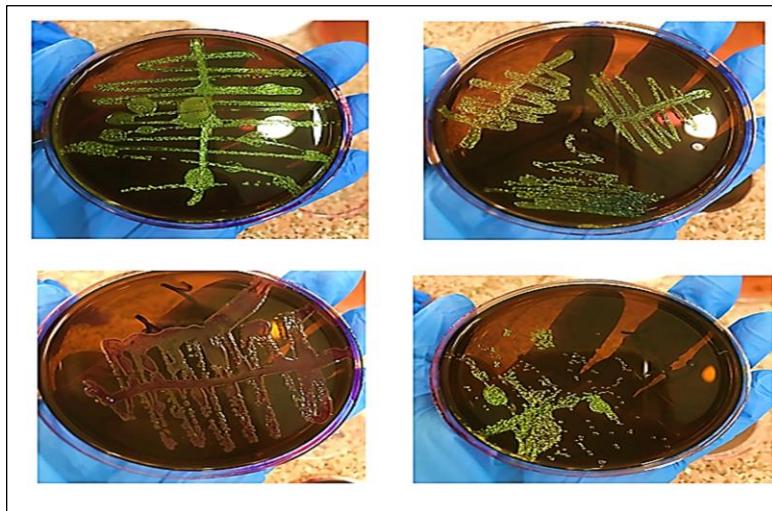
**Figure 1. DBD plasma jet. (a) Single ring electrode configurations (b) Schematic of the DBD plasma jet.**  
**Eosin Methylene Blue (EMB) Agar**

Exact 36.56 grams of EMB Agar (Manufacturer : HI Media Laboratories, INDIA) was dissolved in 1000 mL of distilled water. Then, it was mixed uniformly and heated to boil to ensure completely dissolved process in the media. The media was sterilized by autoclaving at 15 lbs pressure and 121°C for 15 minutes. This media was cooled at ranged 45-50 °C and shake to oxidize the methylene blue (e.g., to restore its blue color and to suspend the flocculent

precipitates). The final media was mixed well and pour into pet plates. If EMB Agar is to be inoculated on the same day, it may be used without autoclave sterilization. After all these steps, the media is completely prepared [17]. Numerous scientific investigations necessitate quantitative assessment of bacterial populations, a critical aspect of microbiological research and environmental studies. The two predominant methodologies employed for quantifying bacterial numbers are the standard, often referred to as the viable, plate count method and the spectrophotometric analysis, also known as turbid-metric analysis. While these two approaches exhibit some similarities in the outcomes they produce, it is imperative to recognize that there are noteworthy distinctions between them. For instance, the standard plate count method serves as an indirect measurement of cellular density and provides insights that pertain exclusively to viable, or living, bacterial cells. In contrast, the spectrophotometric analysis hinges upon the principle of turbidity and serves as an indirect measure of the entire bacterial population, encompassing both living and dead cells, thereby evaluating the total cell biomass present in the sample [18].

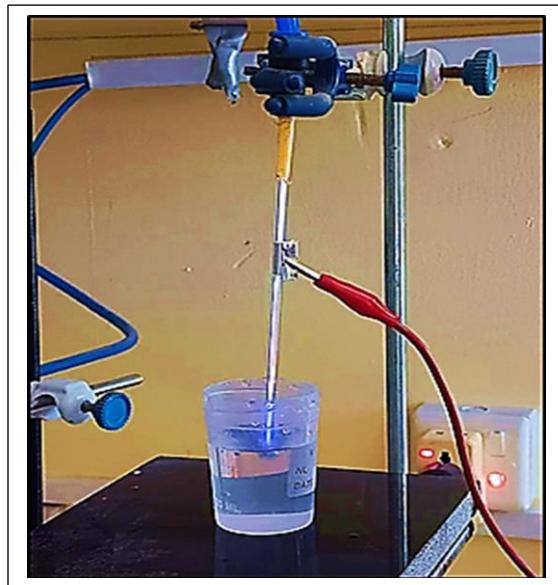
### **Escherichia Coli Bacteria Suspended in Water**

Cultures of *Escherichia coli* O157:H7, a gram-negative bacterial strain that releases Shiga toxin and causes severe foodborne illness when ingested [17]. Using a little CFU from Peter's dish of *E.coli* bacteria (see in Figure 2) to prepare the water samples with *E.coli* by using a single-use loop (Manufacturer : Acost Medical, China). The plastic cap is 60 mL, and set 50 mL of water in the cap, also saving a constant level for all experiments. The sample was well mixed with water in a sterile and special place, such as the work cabin on bacteria in the laboratory and in the presence of a burner to keep the general atmosphere sterile, which gives the number of  $216 \times 10^5$  CFU/mL cells for the first sample, and the second sample is  $235 \times 10^{10}$  CFU/mL . The procedure is explained in Figure 3.

Figure 2. Steps to contaminate water with *E. coli*Figure 3. Steps to contaminate water with *E. coli*

### Treatment of *E. coli* bacteria

The procedure of treatment of *E. coli* bacteria by atmospheric pressure plasma jet was summarized as placing the plastic tube under the plume of plasma jet, where the end of jet immersed 2cm in the water sample. The treatment was taken place for different periods (5, 10, 15, 20 and 25) minutes, as shown in the Figure. 4, for each sample, the working conditions are a gas flow rate of 4L/min and 9kV applied voltage.



**Figure 4.** Treatment of water containing *E. coli* bacteria with cold plasma

The sterilization process was caused increasing the turbidity in a bacterial culture serves as an alternative indicator of bacterial proliferation and total cell numbers, commonly referred to as biomass. By employing a spectrophotometer, it observes that the amount of light transmitted through the culture diminishes as the cell population density increases. This reduction in transmitted light was converted into electrical energy, which is subsequently displayed on a galvanometer, a device that measures electric current or voltage. The resulting measurement, known as absorbance or optical density, serves as an indirect reflection of the bacterial population present within the sample[19]. The water contamination by bacteria was estimated using the spectrophotometer (Manufacturer : Golden Bird, China). Table 1 shows the reduction in turbidity of water sample contains *E.coli* bacteria as the period of treatment is increased. It is clear that using cold plasma in activation *E.coli* bacteria is promising tool.

**Table 1.** Treatment of *E.coli* bacteria by cold plasma at different periods

Time	Control	5 min	10 min	15 min	20 min	25 min
Sample 1	1.87	1.59	1.02	0.9	0.17	0.09
Sample 2	2.70	2.19	1.75	1.342	1.195	0.90

The standard plate count method is a meticulous process that involves diluting a bacterial sample using sterile saline or a phosphate buffer diluent until the concentration of bacteria was sufficiently low to permit accurate enumeration. Specifically, the final plates within the dilution series (see Figure 5) should ideally exhibit a range of 30 to 300 colony-forming units (CFUs) for reliable statistical interpretation. A count of fewer than 30 colonies is deemed unacceptable for statistical analysis due to the possibility that such a small number may not adequately represent the sample as a whole; while a count, exceeding 300 colonies on a single plate is likely to result in colonies that are too closely clustered together, rendering them indistinguishable as separate colony-forming units. The underlying assumption of this method for each viable bacterial cell was separated and distinct from all others, and that each will proliferate into a single, identifiable colony or CFU. Consequently, the resultant number of colonies on the plate was indicative of the number of bacteria capable of growth under the specific incubation conditions that have been employed [20].

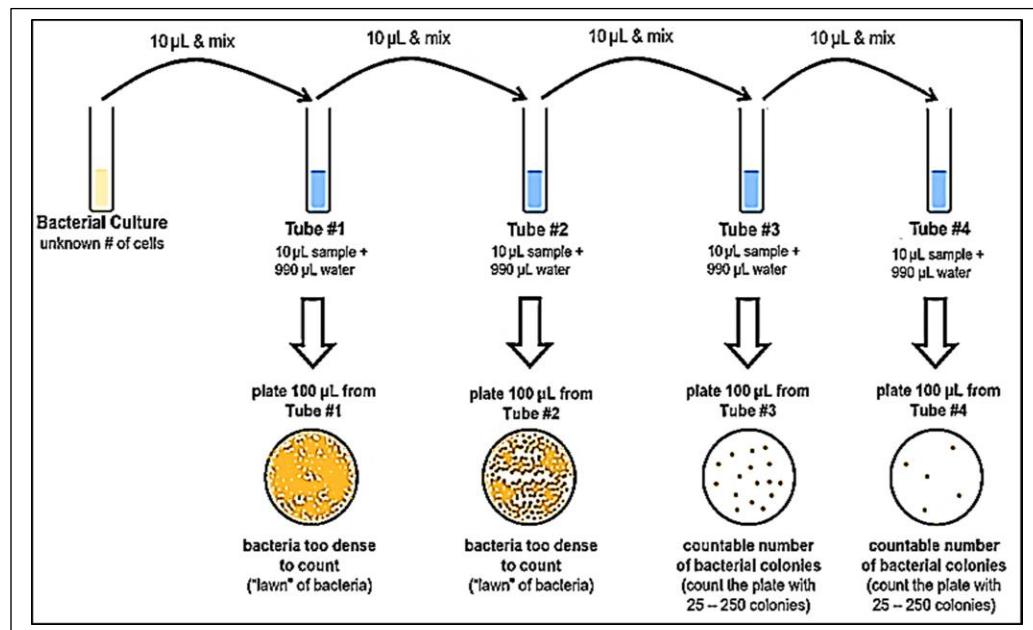


Figure 5. The process of diluting a bacterial culture in a dilution series. When 10  $\mu$ L of the culture is diluted in 990  $\mu$ L of water and mixed [20].

Following plasma treatment of the water samples for different periods, 1 mL aliquots was aseptically pipetted (Pasteur pipette) into Petri dishes for bacterial enumeration. Subsequent serial dilutions were performed to facilitate manual colony counting through a series of steps:

❖ **Plate Count Agar (Standard Methods Agar)**

The 23.5 grams of Plate Count Agar was Put in 1000 mL purified/distilled water. Then it was heated to boiling to ensure the media was completely dissolved. The media was sterilized by autoclaving at 15lbs pressure and 121°C for 15 minutes, and then Cooled to 45-50°C with mixing well and pour into sterile Petri plates(see in Figure 6).

$$\text{PCA} = 23.5 \text{g} \times 500 \text{ mL} / 1000 = 11.75 \text{ mg}$$

Thus, 11.75 mg of plate count Agar (PCA) (Manufacturer : HI Media Laboratories, INDIA) [21] was dispensed into a 500 mL flask. Four identical flasks were prepared to perform serial dilutions across ten tubes, with each dilution series completed within 5-minute intervals following plasma exposure of the samples.



**Figure 6. The Pour the media into the plate (in the lab of microbiology)**

Following a 5-minute plasma treatment of the sample, a serial dilution series was prepared using ten sterile test tubes. The initial transfer was involved aseptically pipetting 1 mL of the treated sample into the first tube containing 10 mL of sterile distilled water, which was

subsequently vortexed for homogeneous mixing. A ten-fold serial dilution series was then established by sequentially transferring 1 mL from the first diluted sample into 9 mL of sterile water. From each dilution tube, aliquots were plated onto nutrient agar plates using the pour plate technique [22-23] (see Figure 6). The plates were gently rotated in clockwise and vertical motions to ensure uniform microbial distribution. This plating procedure and dilutions were repeated at different periods (10, 15, 20, and 25 minutes) for all experimental samples. All plates were incubated (country of origin: Germany) inverted at 37°C for 24 hours. Post-incubation, the plates were examined for colony formation. Colony-forming units (CFUs) were quantified using an automated bacterial colony counter to determine viable cell counts.

## Results and Discussion

### Inactivation of *E.coli* bacteria

Logarithmic reduction in the cellular population of bacteria observed as a direct function of the duration of plasma treatment applied to water. In this context, it is essential to highlight that the bacterium *Escherichia coli* exhibits a notable sensitivity to helium plasma, which consequently results in a significant effect that can be quantified and analyzed. The extent of this bactericidal effect is intricately linked to the duration of the plasma treatment applied. It has been demonstrated that an increase in both the duration of plasma processing and the power input significantly enhances the overall efficacy of the inactivation procedure. Specifically, the investigation reveals that the complete inactivation of bacteria was achieved after a treatment duration of 25 minutes when the flow rate inputs were set  $4L/min$ . During this period, the initial bacterial population exhibited a reduction from  $216 \times 10^5 CFU/mL$  to  $190CFU/mL$  for the first sample (the bacterial count is  $216 \times 10^5 CFU/mL$ ), while the second sample (the bacterial count is  $235 \times 10^{10} CFU/mL$ ) displayed a decrease from  $235 \times 10^{10} CFU/mL$  to  $4 \times 10^2 CFU/mL$ , are the direct results obtained using the counting device described in Figure 7. Furthermore interesting is the observation of inactivity of *E.coli* in the first sample in the first five minutes  $216 \times 10^5 CFU/mL$  to  $75 \times 10^5 CFU/mL$  (see Figure 8); However, the effectiveness of this treatment was lower than

that observed at the same time for the second sample  $235 \times 10^{10} CFU/mL$  to  $42 \times 10^8 CFU/mL$  (see Figure 9). These results highlight the important interaction between treatment length and flow rate inputs in obtaining optimal bacteriostatic results in many applications related to microbial control systems and water purification [24].

Plasma-induced *E.coli* inactivation primarily occurs through:

1. Reactive Species Penetration: He-plasma generates ROS/RNS (OH,  $O_3$ ,  $NO_X$ ).
2. Physical Damage: UV photons and charged particles cause DNA strand breaks.
3. Synergistic Effects: Longer exposures ( $\geq 15$  min) enable cumulative damage overcoming bacterial repair mechanisms.
4. Concentration Dependency: Higher initial bacterial loads (Sample 2) require extended treatment due to quorum effects and antioxidant production.
5. Hydrodynamic Optimization: The 4 L/min flow rate maximizes radical transport while minimizing thermal denaturation [25, 26].

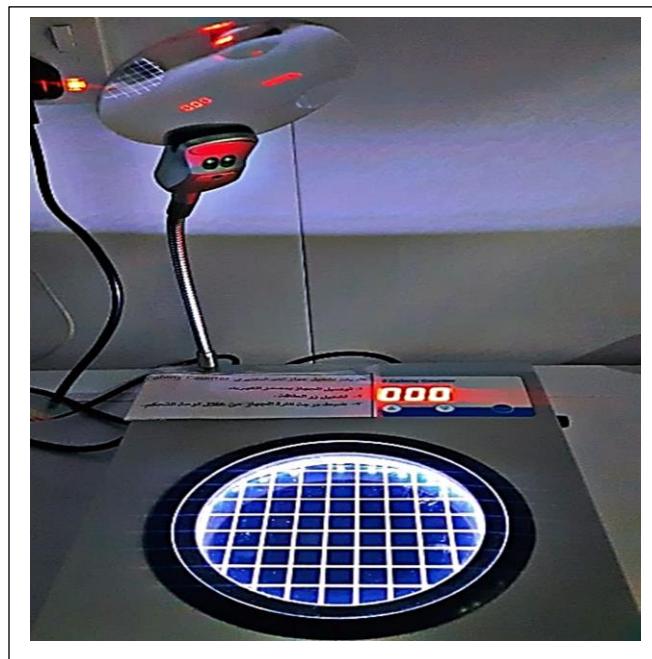
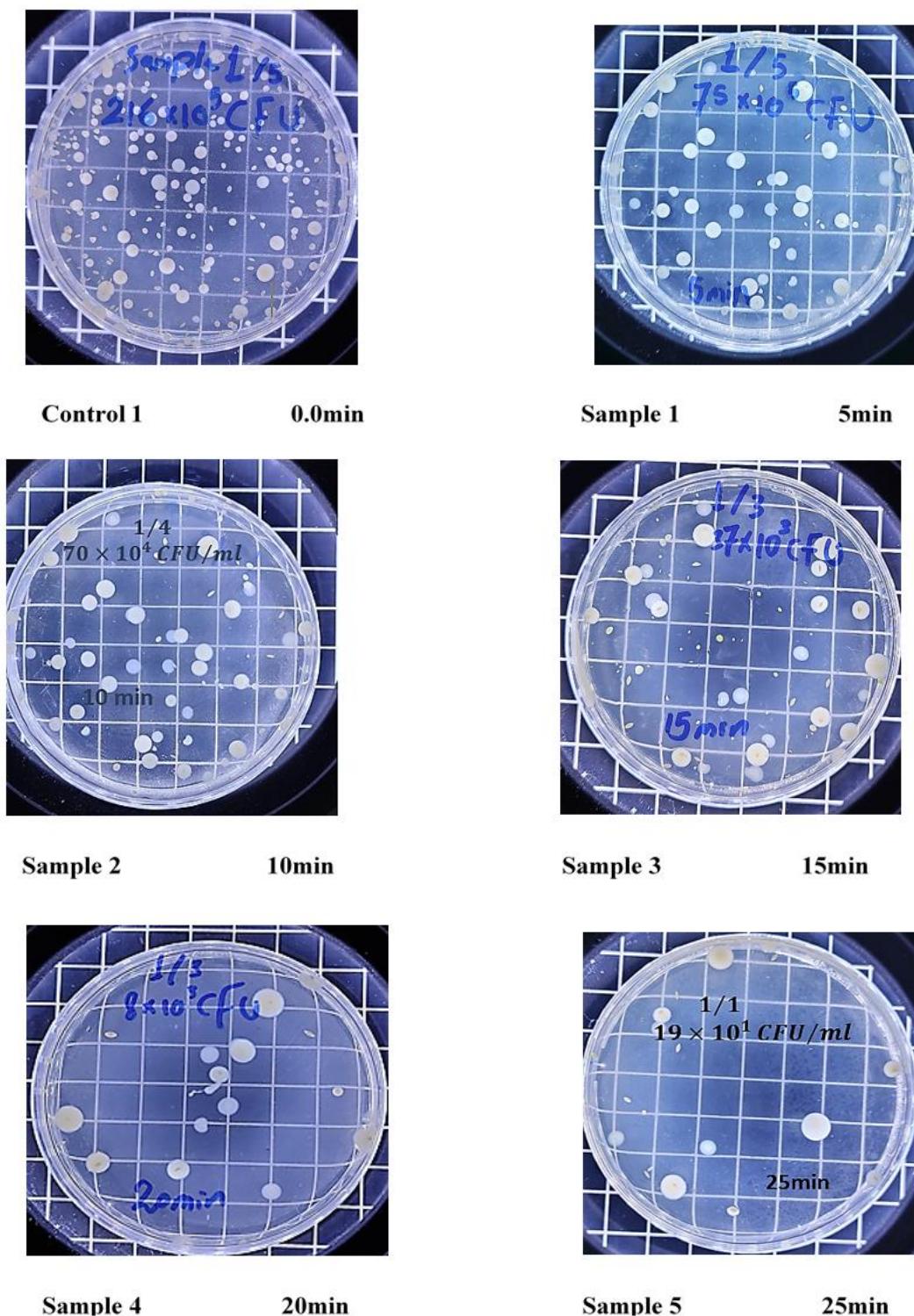
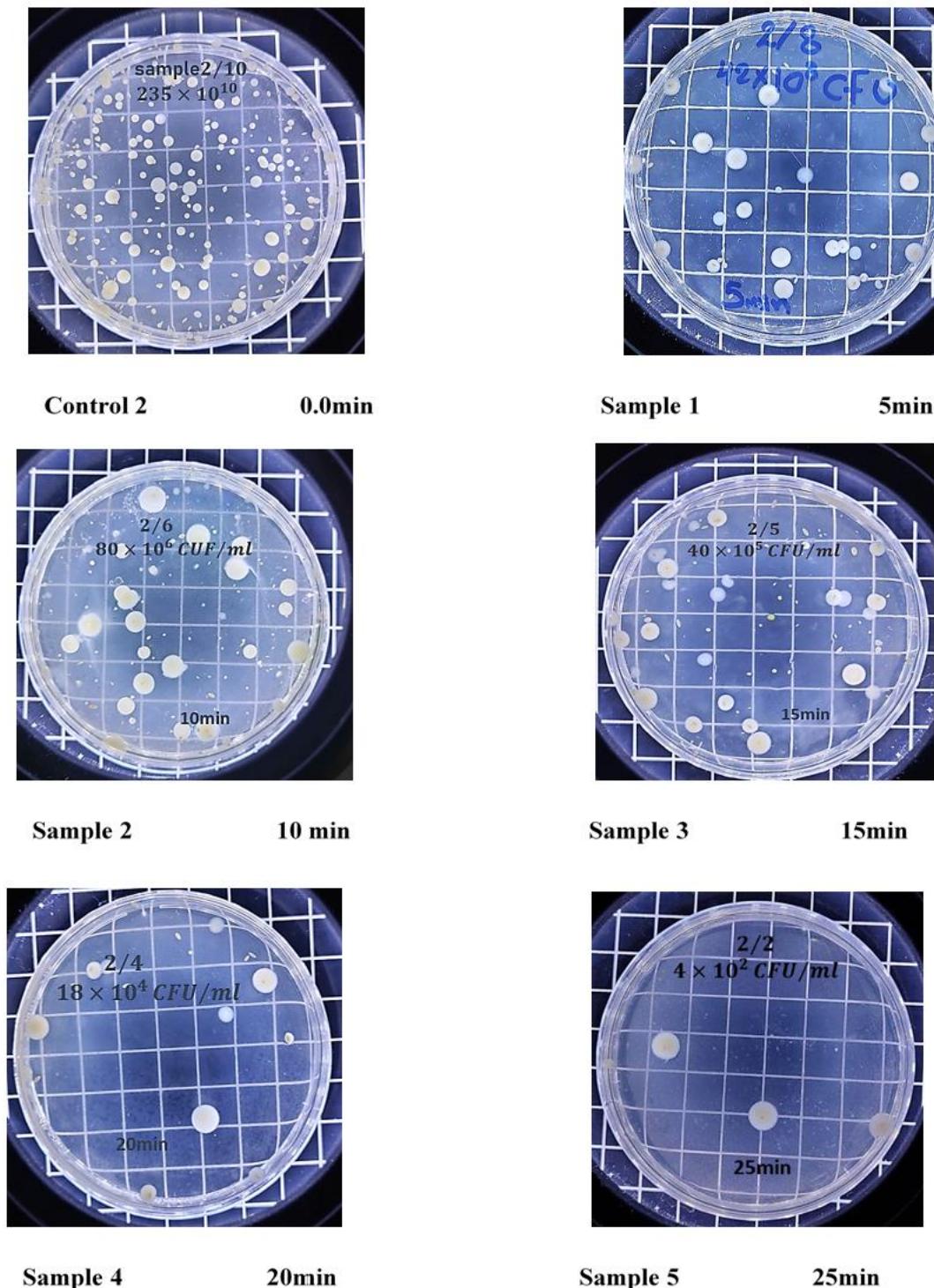


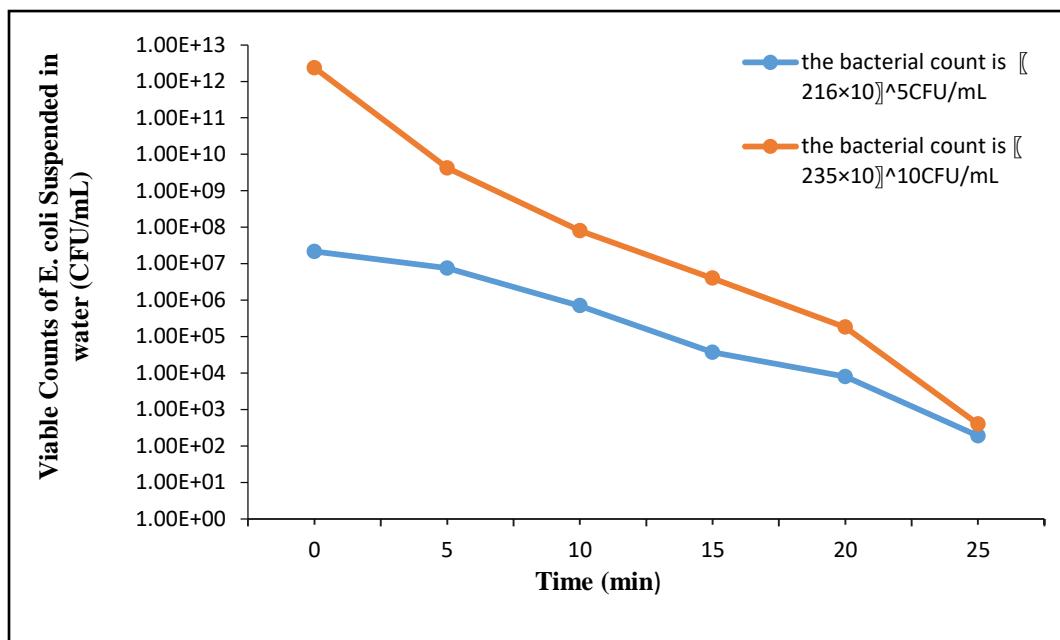
Figure 7. Bacterial counting device



**Figure 8. Viable Counts of Gram-Negative *Escherichia coli* (the Bacterial Count is  $216 \times 10^5$  CFU/mL) at Different Time Intervals (5, 10, 15, 20, and 25) minutes Following Treatment with Cold Atmospheric Pressure Helium Plasma (Flow Rate: 4 L/min).**



**Figure 9. Viable Counts of Gram-Negative *Escherichia coli* (the Bacterial Count is  $235 \times 10^{10}$  CFU/mL) at Different Time Intervals (5, 10, 15, 20, and 25) minutes Following Treatment with Cold Atmospheric Pressure Helium Plasma (Flow Rate: 4 L/min).**



**Figure 10. The effect of using plasma jets under atmospheric pressure on the number of *Escherichia coli* bacteria suspended in water at different times.**

## Conclusion

An active chemical species is formed during the interaction of plasma jet columns with contaminated water. Reactive oxygen species (ROS) and hydroxyl radicals are major sources of oxidative stress on *E.coli* cells. ROS can damage the cytoplasmic membrane and disrupt cellular DNA. By adjusting the plasma input from the flow rate to 4L/min, the concentration of oxygen species increases. The water temperature reaches approximately 28 °C after 5 minutes equaling the temperature of the plasma column at 9 kV applied voltage. The inactivation of *E.coli* bacteria by an atmospheric pressure plasma jet is a promising tool based on the results presented in this work. The reduction in turbidity of the *E. coli* bacteria containing water samples increases with treatment time (from 1.87 to 0.09 for Sample 1 and from 2.70 to 0.9 for Sample 2) at period treatment of 25 minutes. Additionally, the initial bacterial population exhibited a reduction from  $216 \times 10^5$  CFU/mL to 190 CFU/mL for the Sample1, while the Sample 2 showed a reduction from  $235 \times 10^{10}$  CFU/mL to  $4 \times 10^2$  CFU/mL. Furthermore interesting observation is the inactivation of *E.coli* in the Sample 1 within 5 minutes (from  $216 \times 10^5$  CFU/mL to  $75 \times 10^5$  CFU/mL).

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