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Inactivation of *Escherichia coli* by atmospheric pressure plasma jet in water

Mehdi Bakhshzadmahmoudi [a,*], Nader Morshedian[a], Ahmad Mehramiz[b] and Manijeh Kharaghani[b]

[a] Plasma and Nuclear Fusion Research School, Nuclear Science and Technologies Institute, Tehran, Iran
[b] Department of Physics, Faculty of Science, Imam Khomeini International University, Qazvin, Iran
*Corresponding author. E-mail: Bakhshzad@gmail.com

MB, 0000-0002-5574-3747

ABSTRACT

The main aim of this work is inactivation of *Escherichia coli* in water using a laboratory-scale radio-frequency atmospheric pressure Argon plasma jet. This bacterium is widely present in the environment, especially in drinking water, and its pathogenic effects are very harmful. For this purpose, an Argon flow rate of 3.5 slm, maximum plasma power of 200 W, and discharge frequency of 13.56 MHz was conducted to generate a uniform plasma plume for water treatment. 150 ml of drinking water contaminated by *E. coli* was exposed to the radiation of plasma placed about 3 cm within the water, the treatment time varied from 2 to 6 minutes at 100, 150, and 200 W of plasma input power. The temperature of the plume, discharge current and voltage, and electron density were all measured to characterize the plasma. Active species such as excited molecules, ions, and radicals produced in the plasma in water were detected using the optical emission spectroscopy method. The decreasing behavior of live bacteria versus exposure time and plasma jet input power was observed, and finally, at the discharge power of 200 W and 6 min, an effective inactivation was achieved and the number of bacteria reduced from 92×10^4 to less than 1.7 MPN/100 ml.

Key words: Argon plasma jet, atmospheric pressure plasma, drinking water, *E. coli* inactivation, plasma spectroscopy, RF plasma jet

HIGHLIGHTS

- Construction of a 200-W atmospheric pressure Argon plasma jet.
- Using a 13.56-MHz radio-frequency power supply for plasma generation.
- Treatment of water by a stable Argon plasma plume.
- Inactivation of *Escherichia coli* in water by plasma.

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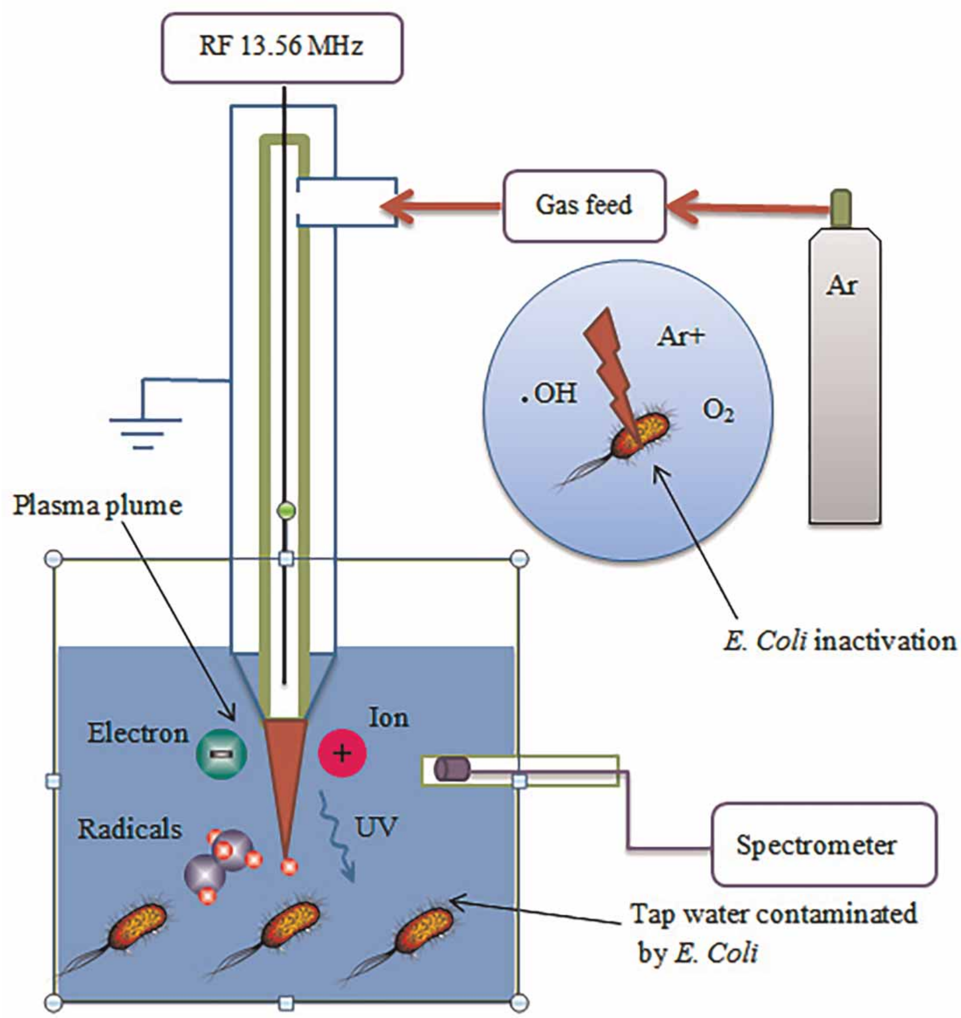
GRAPHICAL ABSTRACT



1. INTRODUCTION

The declining clean water resources have always been a global concern. Climate changes and a greater world population create a greater demand for freshwater; so, new technologies are needed to recycle water more economically. Companies supplying drinking water are seeking for cost-effective, quick, and environmentally-friendly alternatives to provide safe drinking water. Plasma technology offers a new solution for decontaminating the already used water. Plasma generates reactive species, electrons, ions, and radicals which have great effects on the biological and chemical properties of materials.

In recent years, the interaction of various types of plasma with contaminated water was investigated. One of these sources is a non-thermal atmospheric pressure plasma jet that is produced in a dielectric barrier discharge (DBD) structure and uses rare gases like Argon or Helium (Machala *et al.* 2012). The plasma plume can be generated by different kinds of power supplies, such as pulsed DC, AC, RF, and microwave. The plasma produced in this way generates intense UV radiation, shock waves, and active chemical radicals like hydroxyl (OH), atomic oxygen, and reactive oxygen species. These agents are useful for decontamination since they have a limited lifetime (Bruggeman & Leys 2009). The OH radical has a corrosive impact on cellular membranes. When the plasma plume is out of the liquid or its tip is in touch with the liquid's surface, the plasma jet interacts with it in several ways (Vanraes *et al.* 2016). The interaction mechanism of plasma within the water is less understood than outside water. Inactivation of *Escherichia coli* by using atmospheric plasma jet has attracted considerable

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attention from researchers (Niemira *et al.* 2018; Asghar *et al.* 2021; Liu *et al.* 2021). *E. coli* is a rod-shaped, Gram-negative bacterium that can be found in the intestines of animals and humans, and contaminates water.

The objective of this study was to investigate the antibacterial effect of atmospheric pressure Argon plasma jet generated by a radio-frequency (RF) power supply on contaminated water by *E. coli.* In fact, the plasma plume was placed at the center of 150-ml liquid volume during the treatment process. Various plasma discharge characteristics such as input electrical power, plasma plume temperature, its optical emission spectrum in water, and electron density were evaluated in order to assess the plasma's performance and optimize the process. The results of the interaction of plasma with water contaminated by *E. coli* were investigated with respect to chemical radicals generated in water.

2. EXPERIMENTAL

2.1. Plasma source configuration

A dielectric barrier discharge structure is used for generating plasma jets. The plasma jet and the plasma plume are schematically shown in Figure 1. The device is composed of a central needle electrode with a diameter of 1 mm which is made of stainless steel and inserted in the center of a Quartz capillary rod of 2 mm inner diameter as a dielectric between anode and cathode. In this manner, the gas flows between the internal electrode and the dielectric tube. The central parts are covered by a Teflon tube with an internal diameter of 7 mm, and then it is covered by a grounded outer stainless steel electrode. The central electrode is driven at a frequency of 13.56 MHz. As the RF generator is designed to operate in a 50-Ω load, a matching box is placed between the jet and the RF generator to transform the resistive and capacitive characteristics of the plasma to 50 Ω. Thus, the load impedance and the RF generator impedance are matched.

RF powers were set for 100, 150, and 200 W, and the measurement procedure is described in subsection 3.1. Moreover, a mass flow controller set an Argon flow rate of 3.5 slm. A stable plasma plume with a diameter of 2 mm and a length of 2 cm was created.

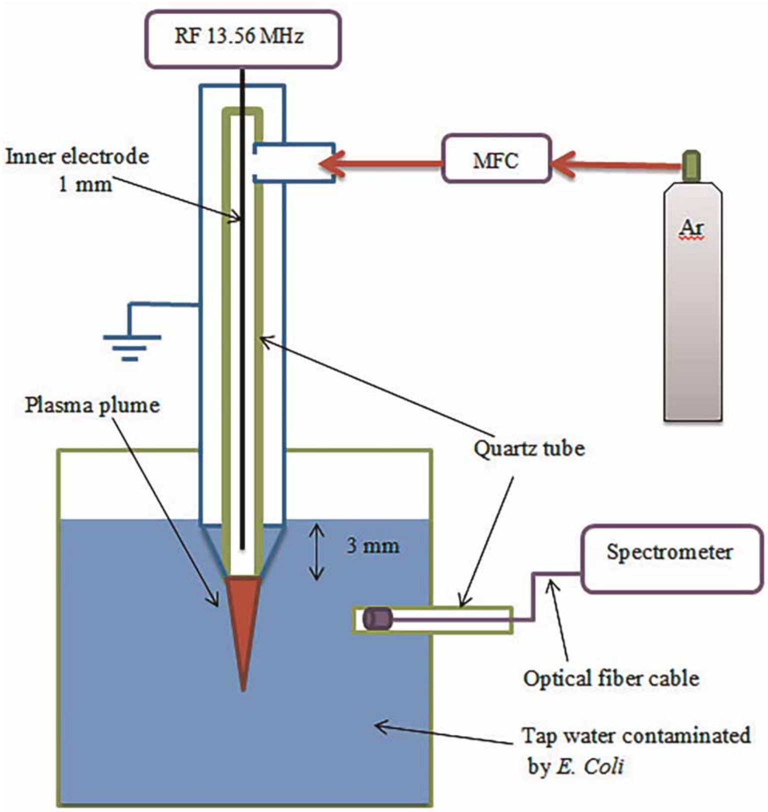

Figure 1 | Schematics of the experimental setup.

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The plasma jet was fixed vertically and suspended directly in water, whereas the plasma plume was placed at the center of the liquid volume in the treatment process (Figure 1). Besides, the inactivation area was not confined to a small volume of the water around the plasma. As the gas flowed into the water continually, the gas bubbles were stirred (Figure 2). Each experiment was performed three times to ensure repeatability. Water contaminated by *E. coli* microorganisms was exposed to the plasma plume for 2, 4, and 6 min. Before and after the treatment, the cells were counted using the multiple tube fermentation technique that provides the Most-Probable Number (MPN) analysis (Baird & Bridgewater 2017). The emission spectrum of the plasma jet in the water was measured to determine the amount of reactive chemical species. For this purpose, an Avantes 4-channel fiber-optic spectrometer with a wavelength of 200–1,058 nm and with a 0.19-nm resolution was used.

2.2. Preparation of contaminated water

Water samples were processed in a 250-ml glass bottle reactor. The water volume was set to 150 ml and kept constant for all the experiments. The cultures of *E. coli O157:H7*, a rare, specific strain of *E. coli* that releases a powerful toxin and will cause severe illness when swallowed, were conditioned for 18 h at incubation state and transferred in Tryptic soy broth (TSB) at 35 °C. Then, the species were centrifuged at 4,500 rpm for 30 min, and after that they were washed using phosphate-buffered saline (PBS). Bottles were refilled with an appropriate amount of tap water to reach 150 ml to give a cell number of 92×10^4 MPN/100 ml.

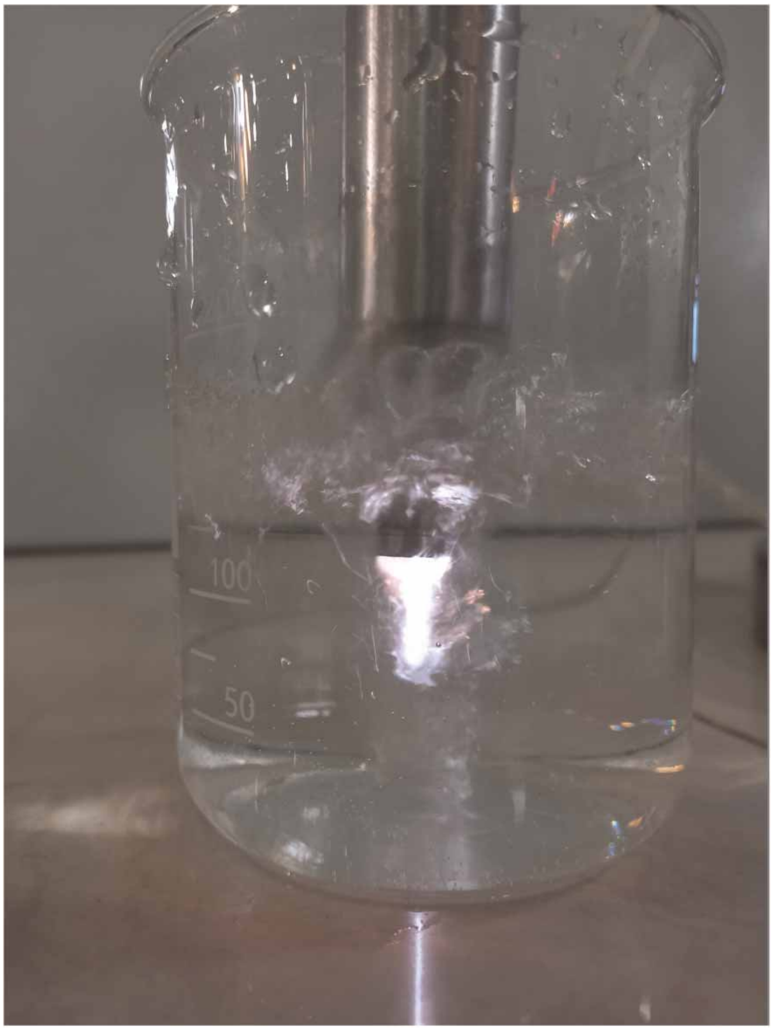

Figure 2 | Treatment of contaminated water by Argon plasma jet.

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3. RESULTS AND DISCUSSION

3.1. Electrical discharge characterization

Measuring the waveform of the applied voltage and current of a plasma source is a convenient way to explain the electrical properties of a dielectric barrier discharge. Electrical behavior was studied via a high-voltage probe (P6015A) and a current probe (4997).

The plasma discharge voltage and current waveforms are shown in Figure 3. They feature sinusoidal forms with a frequency of 13.56 MHz and are proportionate to the power-generating output. The waveforms show that the micro discharges are distributed evenly and consistently. The absence of spikes in the current waveform shows the existence of normal glow discharge (Yanguas-Gil *et al.* 2007). The red line indicates the applied voltage that has a peak-to-peak value of about 800 V. The blue line represents the current, and its peak-to-peak value is about 1 A. The current leading the voltage indicates a capacitive discharge. The dielectric barrier layer which was employed among the electrodes prevents the occurrence of arc at high breakdown voltages. Thus, a uniform glow discharge was formed around the anode electrode (Hofmann *et al.* 2011).

3.2. Plasma plume and water temperature measurements

The plasma temperature was measured in the air by a digital thermometer (JUMO) with a ceramic sensor (PT-100) outside water. The tip of the sensor is covered by glass, which avoids the arc generation between the anode and the thermocouple. The experiments were carried out with the same gas flow and plasma condition, on and off time in the powers 100, 150, and 200 W in the air. In Figure 4, the variation of temperature with time is shown. After running the thermometer, the room temperature was measured at about 27 °C. The plasma was subsequently turned on at t=5 s, the temperature of the thermal probe rose, and the plasma was turned off at t=65 s, with the temperature of the plume almost steady. Therefore, the thermal probe began to cool down. After about 200 s, the temperature reached near to the start point again. The maximum temperatures of the plasma plume were 56.47, 43.55, and 33.61 °C at 200, 150, and 100 W, respectively.

The interaction of plasma with water warms the water by energy transfer. The temperature of the water was determined by a thermometer after completing the plasma treatment. Figure 5 illustrates the variation of water temperature with time at different input powers.

3.3. Optical characterization

Optical emission spectroscopy is a common method to identify active species generated by the plasma. As the radicals react very quickly, it is necessary to measure them *in situ*. For this purpose, the discharge emission spectrum was determined while

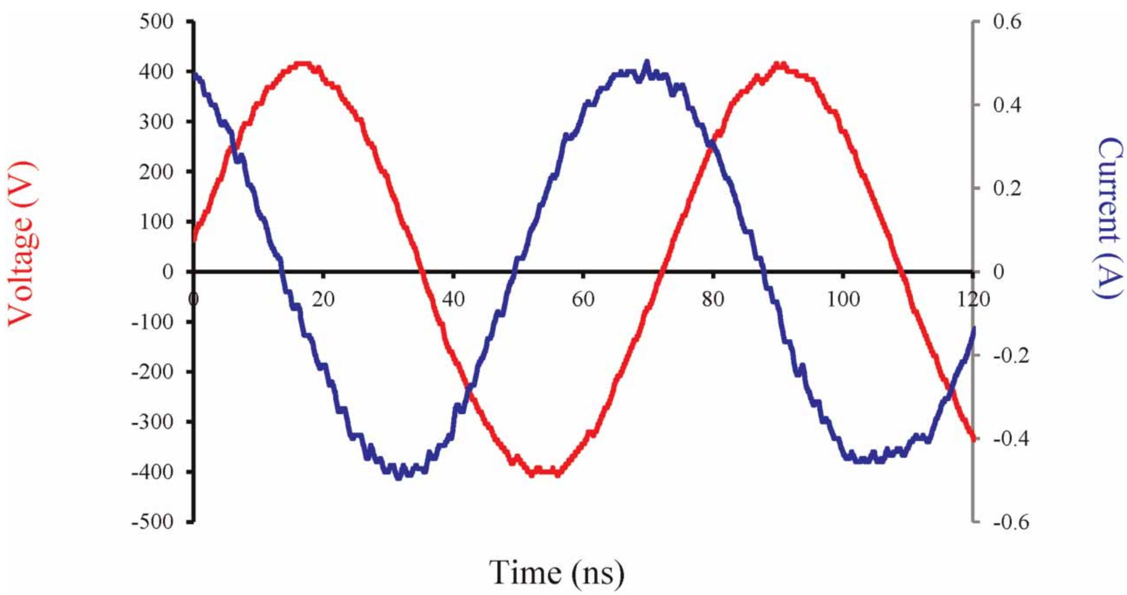

Figure 3 | Waveforms of discharge voltage and current. Please refer to the online version of this paper to see this figure in color: http://dx.doi.org/10.2166/wh.2022.011.

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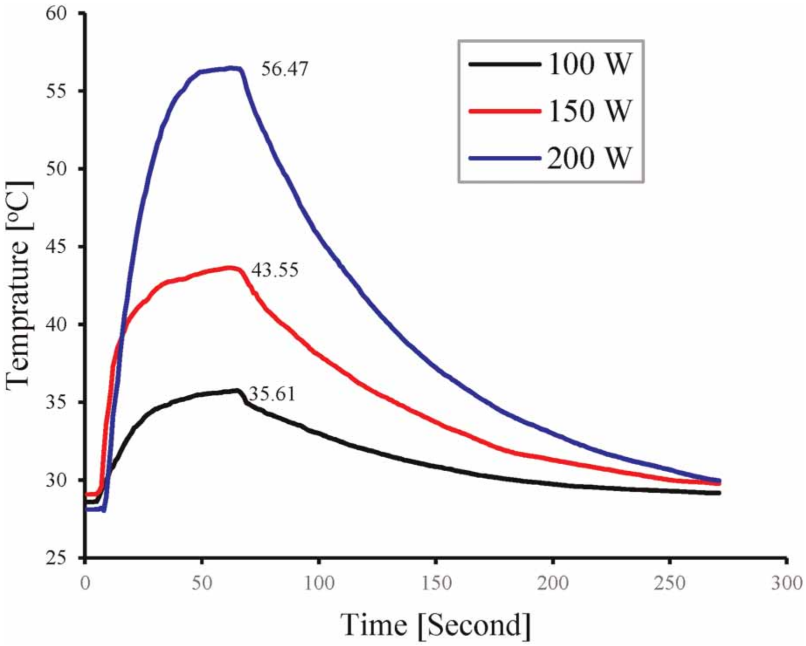

Figure 4 | Plasma plume temperature measurement at different input powers.

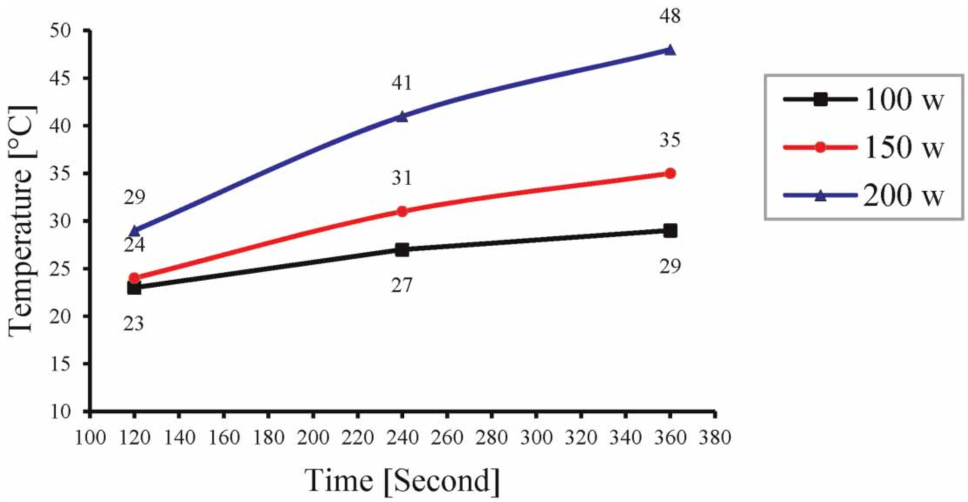

Figure 5 | Temperature of the water treated by plasma at different input powers.

the optical fiber was placed in a sealed quartz tube and placed at a distance of 3 mm from the plasma plume inside water. Figure 6 shows the emission spectrum of Argon plasma plume in water at different powers in the spectral range from 200 to 1,000 nm.

In all figures, intensive Argon lines can be found in the spectral region between 680 and 850 nm (Sarani *et al.* 2012). Besides Argon lines, OH radical species in the 308 nm and O in the 777.4 and 844.6 nm can be identified. The OH emission is due to the fragmentation and excitation of water molecules by high-energy electrons according to Equation (1) and the metastable state as shown in Equation (2) (Hernandez-Arias *et al.* 2012).

$$H_2O + e \rightarrow H + OH + e \tag{1}$$

$$H_2O + Ar^m \rightarrow OH + H + Ar \tag{2}$$

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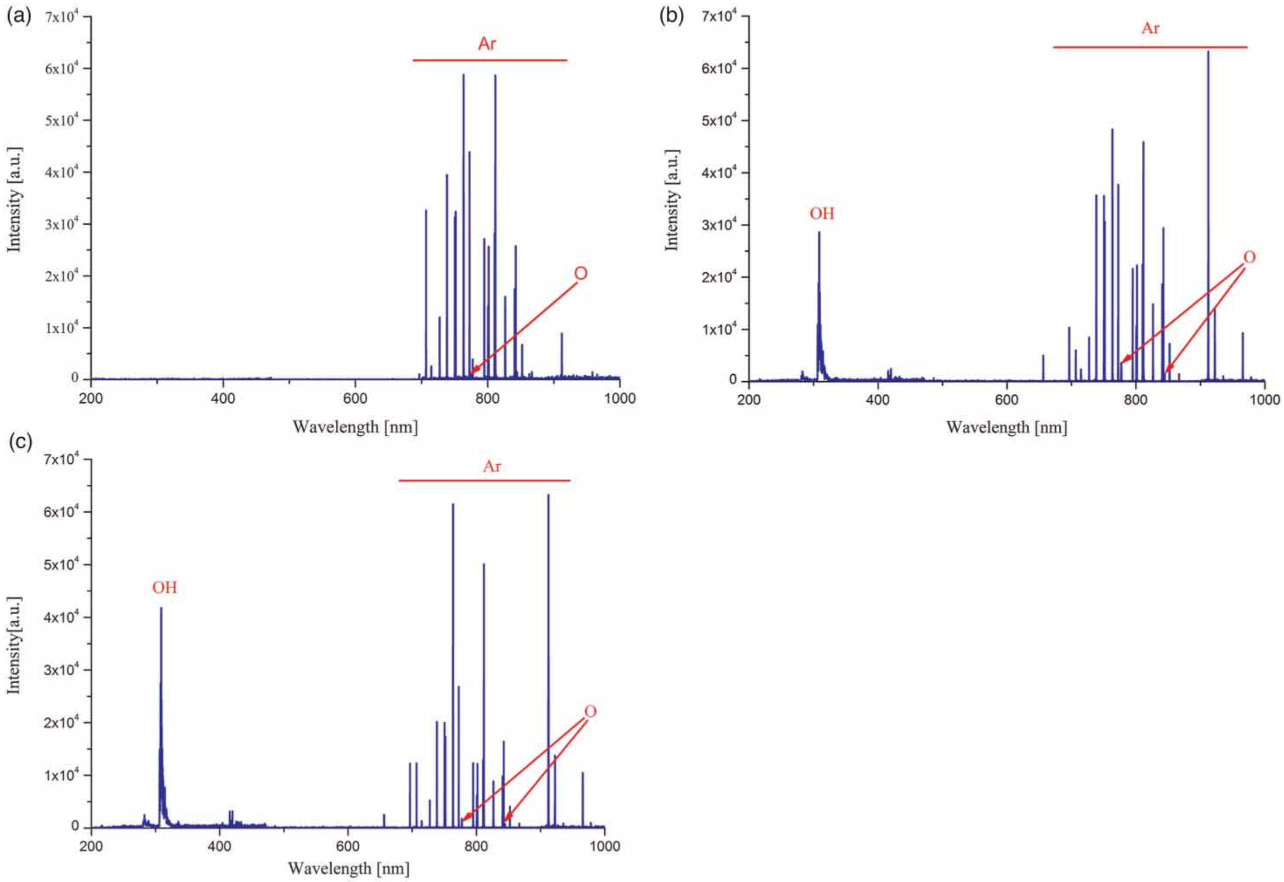

Figure 6 | Emission spectrum of the Argon plasma jet in water at (a) 100 W, (b) 150 W, and (c) 200 W.

It was also found that no emission of OH species happened at 100 W, while by increasing power to 150 W, an emission line appeared, and by increasing the power the line gets stronger.

Furthermore, the interaction of high-energy electrons with the dissolved oxygen in water generates oxygen atoms (Feng *et al.* 2005).

$$e + O_2 \rightarrow O^- + O \quad (3)$$

$$e + O_2 \rightarrow e + O + O(^1D) \quad (4)$$

$$e + O_2 \rightarrow O^+ + O + e \quad (5)$$

Moreover, UV light emission can be observed when plasma interacts with water, especially in 150 and 200 W. This emission is in terms of the relaxation of excited species to lower energetic states (Jiang *et al.* 2014).

3.4. Electron density determination

The Stark broadening analysis was used to obtain the electron density in atmospheric pressure plasma. The broadening is caused by the interaction of Argon atoms with free electrons and ions through the electric field. The broadening of the Hydrogen Balmer line H_b line 486.1 nm gives a good approximation of the electron density (n_e) (Balcon *et al.* 2007). For atmospheric pressure plasma in Argon, the variation of electron density is $10^{11} \leq n_e \leq 10^{17}$ cm^{-3}. The full-width at half-maximum (FWHM) of Stark broadening of the H_b line is related to the electron density as Equation (6) (Qian *et al.* 2010).

$$\Delta\lambda_{\text{Stark}} = 2 \times 10^{-11}(n_e)^{2/3} \quad (6)$$

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where electron density is in the unit of cm^{-3} and $\Delta\lambda_{Stark}$ is in nm. To determine (n_e), a Voigt shape is fitted to the measured lines and the Lorentzian part of the FWHM is used. Figure 7 shows the dependence of the electron density at different powers.

The calculation of the electron densities at different powers is listed in Table 1.

3.5. Bacteria inactivation

Figure 8 indicates the logarithmic cell number decline of the bacteria as a function of plasma treatment time in the water. It indicates that *E. coli* are sensitive to Argon plasma and thus there is a definite bactericidal impact. The size of this impact is determined by the length of treatment and the amount of power used. The survival curve in Figure 8 indicates that increasing the plasma treatment time and power increased the efficacy of the process. In particular, almost a complete inactivation of bacteria was achieved after 6 min at an input power of 200 W, and the number of cells reached from 92×10^4 to less than 1.7 MPN/100 ml. The same result was obtained at the input power of 150 W and the number of surviving cells reached 2 MPN/100 ml in the same period. Also, the inactivation of *E. coli* can be observed at 100 W, which is less effective.

3.6. Analysis

These findings can be interpreted using active chemical species that are formed during the interaction of plasma plumes with the contaminated water. Reactive oxygen species (ROS) and OH radicals are dominant sources of oxidative stress on *E. coli* cells. ROS can damage the cytoplasmic membrane through chemical reactions and make the DNA of the cell vulnerable (Li *et al.* 2013). At 100 W of power, electrons do not have enough energy to generate OH radicals in the plasma region. So, OH radicals are not observed, and the deactivation rate is very slow. Inactivation is achieved after 6 min due to the presence of oxygen species. They kill the bacteria by oxidizing the bacterial cell membrane and thus damaging nucleic acids and proteins

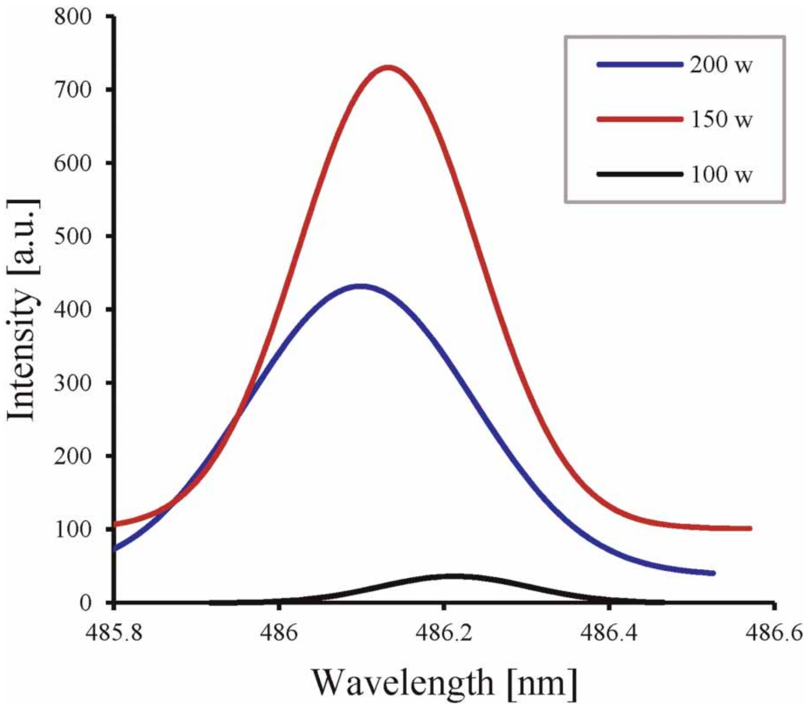

Figure 7 | Voigt function fitting of H_b spectral line at different powers.

Table 1 | Electron density calculation of the Argon plasma jet at different powers

Power (W)	$\Delta\lambda_{Stark}$ (nm)	n_e (cm^{-3})
100	0.2124	1.094E+15
150	0.2562	1.449E+15
200	0.3215	2.038E+15

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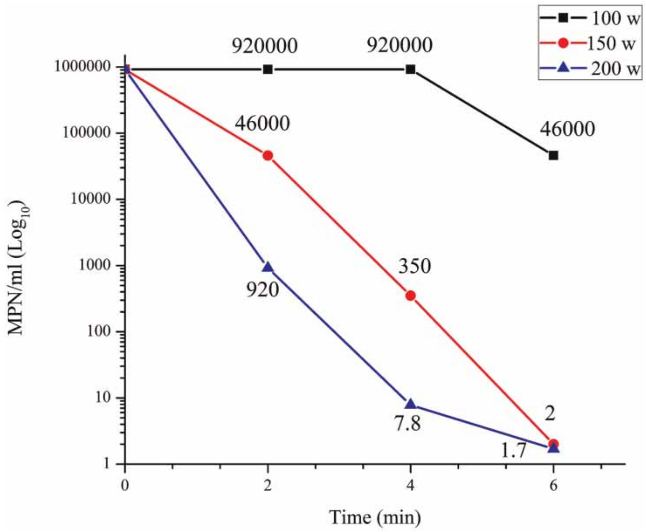

Figure 8 | Number of surviving *E. coli* after plasma treatment at different powers.

within the cell (Lu *et al.* 2016). By increasing the plasma generator power to 150 W, the amount of oxygen species is elevated, and electrons have enough energy to generate OH radicals and the OH line appeared. By increasing the power even more to 200 W, the intensity of the radicals is also elevated which can effectively increase the rate of cell inactivation.

Besides, UV radiation emitted by plasma, especially in 150 and 200 W, can inactivate bacteria. According to Figure 6 and measurement of UV radiation of plasma jet from 200 to 400 nm in water, it can be found that at 308 nm, there is a significant intensity of UV that can have bactericidal effects (van Gils *et al.* 2013). Therefore, these results show cytoplasmic membrane damage, which demonstrates the damaging effects of the UV emission of plasma on nucleic acids (Sato *et al.* 2006).

The plasma plume temperature varies with plasma input power. According to Figure 5 and water temperature measurements, after 6 min, the temperature of water reached near the plasma plume temperature in each power. The maximum water temperature in this study was about 48 °C at the maximum power of the experiments. Compared to the literature about the effect of water temperature on damaging *E. coli*, it can be explained that only temperatures above 55 °C can inactivate bacteria, and the effect of heat in this connection is neglected (Murphy *et al.* 2004).

4. CONCLUSION

This study aimed to investigate the effect of plasma plumes on *E. coli* in water. An atmospheric pressure Argon plasma jet was developed with a driving frequency of 13.56 MHz and a maximum power of 200 W. It was clearly observed that plasma treatment could effectively disinfect water contaminated by *E. coli*.

The characteristic of RF atmospheric pressure plasma jet generated in Argon gas and the effect of plasma treatment on *E. coli* in a liquid phase at different time periods and powers were studied. This work provides a better understanding of the agents produced in water when interacting directly with the Argon plasma plume. The high electron density of the Argon plasma jet can produce more radicals as a result of electron impact dissociation.

Active species were successfully characterized using optical emission spectroscopy, while the head of the optical fiber was placed near the plasma plume in water to measure the emitted spectrum exactly. It was observed that through the interaction of plasma and water, the OH radicals appeared in 308 nm at 150 and 200 W. This species had a dominant role in bacterial inactivation under these experimental conditions. It was found that there is a relationship between the appearance of the OH radical and *E. coli* inactivation; by increasing the power of the RF generator, the rate of inactivation dramatically increased and reached less than 1.7 MPN/100 ml after 6 min at 200 W which was in terms of the increase in energy of electrons in producing OH radicals. The second mechanism of plasma sterilization was the presence of reactive oxygen species and oxidation effect as presented in all three spectra. As the entire process was done under 48 °C, the effect of heat on cells was negligible. The next agent was ultra-violet radiation that was produced by plasma discharge in water. In this context, the atmospheric Argon plasma jet has a great potential for the inactivation of water contaminated by *E. coli*.

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DATA AVAILABILITY STATEMENT

All relevant data are included in the paper or its Supplementary Information.

CONFLICTS OF INTEREST STATEMENT

The authors declare there is no conflict.

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