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ABSTRACT
In this work, the bacteria inactivation using the nonthermal atmospheric pressure plasma has been studied. The bacteria inactivation was conducted using a self-design nonthermal atmospheric pressure plasma jet system. During this experiment, *Escherichia coli* was used as an objective microorganism. The primary operating gas for the plasma jet used in this work is helium, and small fractions of oxygen or nitrogen (0.2%) were used as the secondary gas. The three plasma jet cases were operated at 3.5 kV, 14 l/m, and 7 mm, which represented the applied voltage, gas flow rate, and distance from the nozzle, respectively. The types of reactive species have been examined using optical emission spectroscopy. The gas temperature and optical emission spectrum were measured under the same condition. The active species of OH, OII, OI, N$_2$$^+$, N$_2$$^2+$, and He are indented in the UV-vis wavelength range. The inactivation of *E. coli* bacteria has occurred after 20 s of nonthermal plasma treatment, whether the carrier gas is pure helium or helium + nitrogen or helium + oxygen. The results revealed that the impact of helium is less than that of helium + 0.2% nitrogen which is less than that of helium + 0.2% oxygen. The current results of this experiment could be utilized in improving the nonthermal plasma jet for extended surface decontamination.

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I. INTRODUCTION

The plasma is defined as a partially or wholly ionized ambiance, representing a blend of numerous chemical substances, ultraviolet radiation, and heat. According to the gas temperature, the nonequilibrium plasma can be categorized into the thermal (hot) or nonthermal (cold) plasma. However, in the last two decades, various nonthermal atmospheric pressure plasma (NTAPP) designs were examined, which have their application in thermally sensitive substances and biomedical treatments. Currently, it has been observed that the use of the NTAPP is rapidly growing in biomedical research. Due to the increasingly severe obstacle of emerging antibiotic-resistant microorganisms, there is an essential requirement for the design and improvement of unique selections to antibiotics. In this opinion, the NTAPP reproduces a vital and useful alternative method in overcoming resistant microorganisms.

The NTAPP applied for the suppression of hazardous microorganisms and pollutants has latterly created growing interest in the research of biological and chemical sterilization. The NTAPP can be produced either in a vacuum reactor or in the open air at atmospheric pressure, and it is recently widely considered as a powerful low-temperature decontamination technique. The NTAPP can be produced by corona discharge, glow discharge, dielectric barrier discharge, and so on. Despite the ability of the NTAPP to kill pathogens, the mechanisms of plasma inhibition of microorganisms are ill-defined. Recently, the influence of the NTAPP on microorganisms has acquired numerous interests. The NTAPP can be used to sterilize heat sensible therapeutic equipment such as endoscopes, in dentistry, and in dermatology. The NTAPP can be used as an alternative to conventional decontamination procedures of stents and implants, for bacteria inactivation, or for wound healing.
The NTAPP has become a powerful method to efficiently and efficaciously control multidrug resistant microorganisms. According to the plasma types and discharge conditions, the NTAPP is considered as an effective source of a mix of reactive species, electronically excited atoms, charged particles, ozone, UV photons, and radicals. In the case of the NTAPP, reactive species are produced through different collisional pathways, such as electron impact excitation and dissociation. Air plasmas are magic sources of reactive oxygen and nitrogen species (RONS), such as O, O3, OH, NO, NO2, etc. The NTAPP has an excellent potential for use to disinfect biological and packaging materials.

The nonthermal plasma can produce short and long-lived reactive oxygen and nitrogen species in either the gaseous or aqueous medium when initial plasma species interact with a liquid phase. In the last few years, plasma-liquid interactions have acquired enormous interest. The conception of plasma-liquid interactions and, in specific, the transfer of reactivity from the gas to the liquid phase have been highlighted as being of foremost importance for the biological system. These interactions are a mix of physical and chemical components that are produced in the gas phase of the nonthermal plasma. UV, heat, and electromagnetic fields are represented by the physical components. On the other hand, the oxygen and nitrogen reactive species are represented by chemical components. The major components in the plasma treatment of the biological target are the blood plasma and cell culture media. The amount of plasma-induced reactive species in a solution depends on many factors such as the nature of the gas, the gas flow, the applied voltage, the distance between the plasma and the solution, the composition of the solution, and the volume of the solution.

The purpose of the current work is to generate reactive species using a self-design helium nonthermal atmospheric pressure plasma jet system. Furthermore, the influence of the addition of 0.2% oxygen or 0.2% nitrogen in the helium plasma jet features has been investigated. The impact of adding oxygen or nitrogen to helium on the plasma jet gas temperature has been studied. The reactive species of the plasma jet in pure helium or impure helium were studied using optical emission spectroscopy (OES). In addition, the impact of the gas composition of the NTAPP on inactivation of Escherichia coli bacteria has been investigated.

II. METHODS

A. Plasma source

In the current study, the nonthermal atmospheric pressure jet system was designed and used in bacteria inactivation. As can be seen in Fig. 1, the design of the jet system has been described in an earlier study. The high voltage power supply is a commercially available transformer for neon light. The neon power supply is appropriate for the nonthermal atmospheric plasma to produce the plasma jet to reduce the total cost of the system by replacing the expensive radio frequency power supply, which represents the main cost. The output of the high-voltage neon power supply is 30 mA, 10 kV, and 20 kHz, which lie in the range of a very depressed frequency of RF. The input of this power supply is connected to a 220 V, 12 A voltage controller. The voltage controller regulates the primary voltage of the high-voltage transformer. The electrode unit of the jet system is made up of two parallel stainless steel rings, and the two rings have been separated by a Teflon insulator. The two rings have an identical thickness and diameter of 15 mm and 2 mm, respectively. On the other hand, the Teflon disk has a thickness of 1 mm and a diameter of 15 mm. A center hole of 1 mm and 1.2 mm diameter has been created in the two rings and Teflon disk, respectively; into the center hole, the gas is flowing. The external electrode and the internal electrode represent the cathode and the anode, respectively. The primary operating gas for the plasma jet used in this work is helium, and small fractions of oxygen or nitrogen (0.2%) were used as the secondary gas. In this experiment, the gas temperature of the NTAPPJ (NTAPP jet) was measured using a thermocouple.

B. Determination of the reactive species in the gas and liquid medium

An optical emission spectrometer detects photon emission in this region. The spectra obtained provide valuable information about the species present within the plasma and their electron excitation. The emission spectra from the nonthermal atmospheric pressure plasma jet were obtained with a spectrometer, which is composed of a lens joined to a detector by a fiber optic cable. In this work, the spectroscope with the model number of HR4000CG-UV-NIR (Ocean Optics) was used. On the other hand, the reactive species contents in the water samples after exposure to the NTAPJ were also analyzed. The H2O2 concentration is measured using titanyl ions. The NO2− concentration is measured using a Griess reagent, whereas the NO3− concentration is obtained using the Acorn Series ION 6 m (pH/mV/µC meter) nitrate electrode.

C. Sample preparation

In this experiment, to study the impact of gas carrier composition on inactivation of the microorganism, Escherichia coli was used. Several colonies were picked up from an overnight culture of each isolate, suspended into 3 ml of brain heart infusion broth, and then kept for 24 h at 37 °C. However, after 24 h of incubation, the bacterial suspension was diluted with the sterile physiological solution to 5 × 10^6 CFU/ml, which was compared with 0.5 McFarland standard
tubes. However, 0.1 ml of the prepared suspension was spread on the nutrient agar in a standard Petri dish. The number of E. coli bacteria is $5 \times 10^8$ CFU/ml. The bacteria have been spread using a sterile swab. The dishes were stored in an aseptic location to dry for 10 min and then exposed to the nonthermal plasma jet. The bacteria were exposed to the NTAPP jet for different time intervals (20, 40, 60, 80, and 100 s). After nonthermal atmospheric pressure plasma treatment, all samples were incubated for 24 h at 37°C.

### III. RESULTS

Optical emission spectroscopy (OES) has been applied for the characterization and diagnostic of the reactive oxygen species and reactive nitrogen species excited during the plasma treatment for decades. The plume plasma of the present instrument was explored by ocean optical emission spectroscopy to investigate the behavior of atomic and molecular helium and other constituent species. In this work, helium, helium + 0.2% nitrogen, and helium + 0.2% oxygen were used as the carrier gas for running the plasma jet. However, in these three cases, the plasma jet was operated at 3.5 kV, 14 l/m, and 7 mm, which represented the applied voltage, gas flow rate, and distance from the nozzle, respectively. The optical emission spectrum of the NTAPP jet concerning helium as a gas carrier is shown in Fig. 2. In this figure, the active species of OH, OII, OI, N$_2$$^+$, N$_2$2+, and He are indicated in the UV-vis wavelength range. Moreover, for the reason that neither oxygen nor nitrogen is present in the employed gas, the interaction between the helium plasma jet and the surrounding air leads to forming these active species. From this figure, it can be observed that there is no notable UV emission between 200 nm and 300 nm. The spectral bands of the hydroxyl group and nitrogen second positive system can be observed in the UV region exclusively. Typically, the emission bands at wavelengths of 309 and 354 nm correspond to OH and N$_2$2+, respectively. One more recorded spectral band peaking at a wavelength of 309 nm corresponds to the radiation of the hydroxyl radical according to OH($A^2\Sigma^+ \rightarrow X^2\Pi$) transition. The existence of this structure in the spectrum is generated by the dissociative excitation of water particles present in the form of atmospheric moisture. The emission bands of nitrogen molecules in the wavelength range of 300–500 nm of the plasma radiation spectrum have occurred due to the electronic-vibrational transitions of N$_2$2+. However, the emissions from the second positive system (C$^1\Pi_u \rightarrow B^1\Pi_g$) of N$_2$ are mainly produced through electron collisions. From Fig. 2, the spectral lines of OII can only be found in the UV-vis region. Moreover, at 337, 406, and 459 nm, these reactive species can be observed. On the other hand, the reactive species of N$_2$$^+$, OI, and He can be found in the vis region only. The reactive atomic oxygen OI can be detected at 645 and 777 nm. The transition 3p$^5$P $\rightarrow$ 3s$^3$S$^0$ has generated the oxygen band at 777.4 nm. Oxygen atoms are produced either through electron-impact dissociation of oxygen molecules or Penning ionization of nitrogen molecules. The 580, 654, and 677 nm observed peaks represented are the N$_2$ first positive system. Furthermore, the reactive species of helium can be found at 587, 667.5, and 706 nm. However, the emission of helium at 587 nm is a result of the transition 3d$^1$D $\rightarrow$ 2p$^1$P$^0$. On the other hand, the emission of helium at 667.8 nm is a result of the transition 3d$^1$D $\rightarrow$ 2p$^1$P$^0$. The emission at 706.5 nm is a result of the transition from helium (3s$^3$S$^1$) to helium (2p$^1$P$^0$).

An apparent change in the optical emission spectrum was observed when oxygen or nitrogen was added to the plasma jet. The optical emission spectrum of the NTAPP jet with respect to helium + 0.2% oxygen as a gas carrier is illustrated in Fig. 3. From this figure, besides the bands that emerged in pure helium, the OI band has arisen at 844 nm. On the other hand, from this figure, it can be observed that the first negative system of the molecular nitrogen ion (N$_2$1–) has arisen at 391 nm. The addition of a small amount of oxygen to the plasma jet results in an increase in the intensity of OI lines at 645 nm and 777 nm. In addition, an increase in the intensity of OH bands is observed.
of OII lines at 337 nm, 406 nm, and 459 nm has occurred. On the other hand, it can be noted that there is a decrease in the intensity of the other emission bands. Furthermore, the addition of electron-negative gas such as oxygen to the helium plasma led to the production of O and O2 ions by electron attachment mechanisms, which results in the reduction of electron density and consequently the decrease in the intensity of the other bands at a constant voltage.\(^{65}\)

On the other hand, the optical emission spectrum of the mixture of the plasma jet concerning the combination of helium and 0.2% nitrogen is illustrated in Fig. 4. From this figure, it can be observed that as the case of helium + 0.2% O2, the first negative system of molecular nitrogen ions (N2−) has arisen at 391 nm. A higher intensity of the emission of the first negative system of molecular nitrogen ions in the plasma jet spectrum compared to the intensity of the second positive system of neutral nitrogen molecules may indicate that the role played in the formation of ionized nitrogen molecules by the Penning ionization due to the interaction of nitrogen molecules with excited helium atoms is more important than the role of electron-impact ionization.\(^{66}\) However, the intensity of nitrogen first positive and nitrogen second positive systems may be, OH, OI, and OII lines increased. The addition of nitrogen to the plasma generates many positively charged particles (N\(^+\), N\(^2+\), N\(^3+\), N\(^4+\), NO\(^+\), N2O\(^+\), and NO\(^2+\)).\(^{67}\) The produced positively charged particles led to the increase in the electron density and consequently the increase in the intensity of reactive oxygen and nitrogen species bands at the same applied voltage. The absence of N\(^2+\) first negative bands in the case of the helium plasma jet indicates that neither Penning ionization nor charge transfer occurs at this stage.\(^{68}\) An atmospheric-pressure nonthermal plasma jet driven by high frequency alternating current and operating on the N\(^2\) and N\(^2\)/O2 gas mixture is investigated.\(^{69}\) They found that, with rising oxygen percentage, the emission intensity of the lines of N\(^2\) (337.1 nm) and N\(^2+\) (391.4 nm) decreases and that of O (777.4 nm) and O (844.7 nm) increases. The result of the addition of 2.5% O\(_2\) to the He atmospheric cold plasma revealed that\(^{70}\) the addition of oxygen to the helium plasma decreases He molecule emission\(^{71}\) but enhances the intensity level of reactive oxygen radicals.\(^{72}\)

At high-energy electron bombardment in the plasma discharge area, oxygen and nitrogen molecules degenerate into radicals and charged particles as follows:\(^{72}\)

\[
O_2 + e^- \rightarrow O + O + e^-, \tag{1}
\]

\[
N_2 + e^- \rightarrow N + N + e^- . \tag{2}
\]

The bonding energy of N≡N is higher (9.8 eV), but that of O=O is only 5.2 eV. However, compared with O\(_2\), N\(_2\) is challenging to dissociate.\(^{73,74}\) However, by comparing the spectroscopy of the current three cases, the mixture gas of He + O\(_2\) possesses an excess amount of reactive species. This behavior can be attributed to the O radical, which possesses strong oxidizability that equips it with the ability to react with other components in the plasma to generate free radicals or other reactive molecules.\(^{75}\) The influence of the addition of little portions of oxygen about 0.1%–0.5% on the plasma jet features was investigated by Thiyagarajan et al.\(^{76}\) They found that the active zone emission spectra were dominated by OH and helium line intensities, whereas the afterglow region emission spectra were dominated by a range of N\(_2\) line intensities.

However, in this work, to understand the generation of reactive species in deionized (DI) water after treatment using the NTAPP jet and how it varies with the change of the gas mixture, we performed a chemical and electrochemical analysis. H\(_2\)O\(_2\) and NO\(_3^-\) were detected in DI water using chemical analysis, while NO\(_3^-\) was detected with electrochemical analysis.\(^{77,78}\) In this experiment, the distance between the nozzle and the DI water surface is 7 mm. Figure 5 reveals the concentration of the reactive species, which is generated in the DI water after 80s of exposure for He, He + N\(_2\), and He + O\(_2\) NTAPP jets. From this figure, it can be found that the concentration of H\(_2\)O\(_2\) in the DI water after NTAPP jet treatment is 1600 \(\mu\)M, 2500 \(\mu\)M, and 4900 \(\mu\)M concerning He, He + N\(_2\), and He + O\(_2\), respectively. Moreover, the NO\(_3^-\) concentration is 300 \(\mu\)M, 1100 \(\mu\)M, and 1800 \(\mu\)M concerning He, He + N\(_2\), and He + O\(_2\), respectively. On the other hand, the NO\(_3^-\) concentration is 500 \(\mu\)M, 1300 \(\mu\)M, and

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**FIG. 4.** Emission spectra from 200 to 900 nm corresponding to 14 l/min, 3.5 kV, and 7 mm as the He + N\(_2\) mixture flow rate, applied voltage, and distance from the jet nozzle, respectively.

**FIG. 5.** The concentration of reactive species in DI water after exposure with the plasma jet for 60 s at different gas carriers.
FIG. 6. The effect of the gas carrier composition on the plasma jet temperature.

2100 μM, concerning He, He + N₂, and He + O₂, respectively. From this result, it can be noted that the concentration of H₂O₂ inside the water has the highest value when compared to the concentrations of NO₃⁻ and NO₂⁻. The results were shown that the concentration of H₂O₂, NO₃⁻ and NO₂⁻ inside the water treated by helium is less than that treated by helium + 0.2% nitrogen, which is less than that treated by helium + 0.2% oxygen. This behavior can be attributed to the mixture gas of He + O₂, which possesses an excess amount of reactive species in the gas medium.

For the given constant flow rate, the applied voltage, gas flow rate, and distance from the nozzle are 14 l/min, 3.5 kV, and 7 mm, respectively, which equals the gas temperature in the plasma jet, measured on the axis with a thermocouple. As can be seen in Fig. 6, for all three carrier gases helium, helium + 0.2% nitrogen, and helium + 0.2% oxygen, the gas temperature increases continuously for all gases by increasing the operation time. From this figure, it is noted that the temperature is the highest for the operation with helium + 0.2% oxygen, lower for helium + 0.2% nitrogen, and the lowest for helium. This behavior can be observed when exposing time is adjusted from 1 to 2 min. From these results, it can be concluded that for a given discharge parameter gas heating is much more efficient for helium + 0.2% oxygen discharge than for helium + 0.2% nitrogen and gas heating is the lowest in the case of helium. This result agreed with that of Mohamed et al.; they have generated atmospheric pressure molecular gases using nitrogen, oxygen, and air. They have found that the temperature is the highest for the operation with oxygen, lower for nitrogen, and the lowest for air. On the other hand, when the exposure time is adjusted to 3 min, the distribution changes: helium + 0.2% nitrogen conserves its thermal energy more than helium + 0.2% oxygen and helium. Moreover, when the exposure time is increased from 4 to 7 min, the distribution changes again and helium + 0.2% oxygen conserves its thermal energy more than helium + 0.2% nitrogen and helium. From these results, it can be noted that no significant rise in the gas temperature of the plasma jet has occurred when the carrier gas changes. It can be concluded that the current plasma jet is very suitable for treating heat sensitive surfaces.

The effect of the composition of the carrier gas of the NTAPP jet on Escherichia coli bacteria can be seen in Table I. This table shows the effect of the plasma at 14 l/min as the gas flow rate, irradiation time (s) He He + O₂ (0.2%) He + N₂ (0.2%)

| Irradiation time (s) | He  | He + O₂ (0.2%) | He + N₂ (0.2%) |
|----------------------|-----|----------------|----------------|
| 0                    | 5 × 10^8 | 5 × 10^8 | 5 × 10^8 |
| 20                   | 6 × 10^5 | 2 × 10^5 | 4 × 10^5 |
| 40                   | 8 × 10⁴  | 1 × 10⁴  | 7 × 10⁴ |
| 60                   | 9 × 10³  | 2 × 10³  | 5 × 10³ |
| 80                   | 5 × 10²  | 5 × 10²  | 9 × 10² |
| 100                  | 9 × 10¹  | 1 × 10²  | 6 × 10² |

FIG. 7. Photographs of Petri dishes after 24 h incubation at 37 °C showing the effects of the NTAPP jet on Escherichia coli after 20 s of exposure for different gas carriers: (a) He, (b) He + N₂, and (c) He + O₂. The effects of the NTAPP jet after 80s of exposure for (d) He, (e) He + N₂, and (f) He + O₂. D is the diameter of the growth inhibition area.
3.5 kV as the applied voltage, and 7 mm as the distance from the nozzle on the number of survivors of bacteria cells. From this result, it can be observed that the rate of survival of bacteria decreases vastly after 20 s of plasma treatment, which means that the number of living cells decreases rapidly in the case of the three carrier gases. It is well known that heat, UV emission, charged particles, and reactive species, which are produced by the nonthermal plasma, play an essential role in microorganism sterilization. 5 According to the OES results, it can be observed that there is no notable UV emission between 200 nm and 300 nm. The disappearance of these peaks infers that the UV radiation did not contribute to the inactivation process and did not share in any damage that will be caused to the cells of bacteria using this jet.

In this experiment, the treatment temperature was in the range of 29–33.75 °C. Under these conditions, we can avoid the hyperthermia effect occurring in the range of 42–50 °C. 6 Du et al. studied the inactivation effect of the microplasma jet on E. coli using different gases. They found that the inactivation effect becomes enhanced and the effect with different carrier gases is shown as follows: oxygen > air > nitrogen > argon. 7

From Fig. 7, it can be observed that the diameter of the antifungal ring generated by the helium jet plasma is less than that generated by helium + 0.2% N₂ jet plasma which is less than that of helium + 0.2% O₂ jet plasma. This result can be attributed to the increasing occurrence of the reactive species intensity for the helium + 0.2% N₂ jet plasma and helium + 0.2% O₂ jet plasma concerning the helium jet plasma in the case of a gas medium. In addition, it can be observed that the diameter of the ring generated by the helium + 0.2% N₂ jet plasma is less than that of the He + 0.2% O₂ jet plasma. On the other hand, from the OES results, it can be observed that the concentration of the active species in the plasma jet in the case of a gas medium with He + 0.2% N₂ is lower than that with He + 0.2% O₂ as a carrier gas under the same condition, making a low inactivation effect of He + 0.2% N₂ than the He + 0.2% O₂ jet plasma. The diameter of the inhibition zones for E. coli bacteria was determined, and it is shown in Fig. 8 under different times of treatment. It is observed that the inhibition zone diameter exhibits a linear increase with plasma exposure time for the three cases of carrier gases. 5 The inhibition area increases by increasing the treatment time of nitrogen nonthermal plasma jet when it is used to inactivate P. aeruginosa. 8 For sterilizing Staphylococcus aureus, the helium atmospheric nonthermal plasma jet is used by Liu and co-workers. 9 They demonstrated that the sterilizing performance depended critically on the discharge parameter of the applied voltage.

IV. CONCLUSION

A nonthermal plasma jet was designed, and a study was conducted to investigate the influence of reactive species on microorganism inactivation. The type, the concentration, and the intensity of reactive species generated by the nonthermal plasma depend on the composition of the carrier gas. The addition of a small amount of nitrogen or oxygen to helium played a significant role in the change of the intensity of reactive species, and hence, the nonthermal plasma characteristic was influenced. The strong oxidizability of the O radical plays a remarkable role in the enhancement of the reactive species concentration of the He + O₂ NTAPP jet compared with the He + N₂ NTAPP jet. In this experiment, the gas temperature was suitable to treat the thermally sensitive materials. The measurements indicate that the rising of the gas temperature is considered negligible when the carrier gas changes. From the measurement of the concentration of RONS in DI water after NTAPP jet treatment, we concluded that among the stable reactive species, H₂O₂ is formed in large amounts, followed by NO₃⁻ and NO₂⁻. The current design is considered as an ideal system for biological treatments, where it ensures that the hyperthermia effect does not occur. During this work, heat or UV radiation did not participate in sterilization of the bacteria. We can conclude from our study that all three carrier gases (i.e., He, He + N₂, and He + O₂) have the potential to inactivate Escherichia coli bacteria. The bacteria when exposed to the three cases of the plasma showed a decrease in their rate of survival. The effect with three different carrier gases states that the impact of helium + 0.2% oxygen is more than that of helium + 0.2% nitrogen which is more than that of helium. The living cell of Escherichia coli decreased by increasing the exposing time, suggesting that the reactive oxygen and nitrogen species have been playing a significant role in the inactivation process. The current system is suitable for decontamination of the microorganisms.

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