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Research Article
Enhanced Microbial Decontamination Using Non-thermal Low Pressure Argon Plasma Jet

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Abstract

Background and Objective: Atmospheric pressure plasma jet (APPJ) gained great interest due to its effectiveness as selective non-lethal technique with low operational costs. In this study, argon APPJ system was designed and the generated cold plasma was applied in disinfection of microbial cells. **Materials and Methods:** Argon APPJ was generated by blowing argon through capillary metallic tube inserted in alumina and powered by 8-25 kHz sinusoidal voltage waveform. The plasma applied in inactivation of microbes by direct exposure of cell suspension, approximately 10 mm below jet nozzle, for different intervals. Interference of organics in exposure medium, on lethal activity of plasma was investigated. **Results:** APPJ jet induced high levels of reactive oxygen (ROS) and nitrogen species (RNS). Jet length increased with applied voltage and flow rate in laminar mode, but decreased with flow rate in turbulent mode. Percent reduction in living cell count was 98.3 and 94.1%, for *E. coli* and *S. aureus* suspended in water after 30s of exposure, respectively, with 2.7- and 2-folds increase in plasma lethal activity, as compared with LB broth medium. D-values (Decimal Reduction Time) were increased from 34-333, 37-476 and 139-385 s for *E. coli*, *S. aureus* and *C. albicans* in water and complex liquid organic media, respectively. **Conclusion:** Designed argon APPJ system can be used in disinfection of different microbes. Plasma antimicrobial activity drastically decreased in presence of organic matter. The generated plasma can be promising approach for treatment of diseases, especially caused by antibiotic-resistant pathogens.

Key words: Argon plasma, APPJ, antimicrobial effect, *E. coli*, *S. aureus*, *C. albicans*, non-thermal plasma

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Plasma is an emerging field of technology that is interdisciplinary in nature. Recently, atmospheric pressure plasma jet (APPJ), a promising plasma technology working under low temperature, has gained special interest due to its large number of application in medicine and industry. APPJ Low temperature plasma characterized by high versatility of reactive species such as ions, electrons, free radicals, neutral particles and wide spectrum of photons generated from ionized gases[1-3].

According to the thermal equilibrium between reactive species, plasma can be categorized into thermal equilibrium plasma and non-thermal plasma. In thermal plasma, all plasma species (electrons, ions, photons and neutral particles) have the same temperature, while in non-thermal plasma electrons have much higher temperature than other plasma species. Indeed, non-thermal plasma generated at low pressure is called "cold plasma". Recently, many researchers have generated "cold plasma" at atmospheric pressure such as glow discharge, gliding arc discharge, dielectric barrier discharge and plasma jet[4]. The great advantages of atmospheric pressure plasma jets (APPJs) were the main cause behind its use in several applications including; sterilization, wound healing, teeth bleaching, skin treatment, decontamination of spacecraft equipment and surface modifications[5-8]. In comparison with other bacterial inactivation methods, APPJs have many superior advantages; such as safety, convenience, lack of residual toxicity and the potential to sterilize at a relatively low temperature[9]. In addition, cold APPJs can treat temperature sensitive and large-area targets with craters and cavities. Therefore, APPJs plasma become an innovative technology in sterilization and disinfection of contaminated surfaces and wastewater, as well as protection and conservation of materials from microbial contaminants[4,9-17]. Indeed, the effect of cold plasma on microbial and mammalian cells and their components has been described by many scientists[2,18-19].

APPJs have been generated using low frequency (LF) power sources in the range of kHz. The generated APPJs by LF sources have long plasma jets which nominated those plasmas to be used for treating irregular surfaces with hole and cavities. Helium (He) plasma jet is the most stable and easiest APPJ to generate due to its lowest breakdown voltage and lower gas temperature[20]. Economically, helium is costly; therefore, intensive research has been done to find alternative cheaper gases such as argon, nitrogen and air. Unfortunately, air and nitrogen have a higher breakdown voltage compared to argon APPJ. Therefore, argon is the nominated gas to be used instead of helium in many cases for medical and industrial applications. Interestingly, using argon increases generated plasma gas temperature and the possibility for the glow to arc transition, however it has low power consumption, low cost and high sterilization efficacy[21]. Although cold APPJ sterilization has fast germicidal activity, it causes nearly no damage to materials and human cell[22].

The main purpose of this study was to design a simple experimental argon atmospheric pressure plasma jet APPJ. The generated plasma was characterized by electrical, spectroscopic and photographical means. Then, the antimicrobial effects of the APPJ against gram-positive, gram-negative bacteria and a fungal candidate namely; *E. coli*, *S. aureus* and *Candida albicans*, were closely investigated. Finally, emphasis was given to the effect of exposure time as well as the exposure medium on the efficiency of microbial disinfection and decontamination.

MATERIALS AND METHODS

Study area: The study was carried out at Environmental Health Department, Environmental Biotechnology Lab, Imam Abdulrahman Bin Faisal University and Physics Department, Plasma technology Lab, Taibah University from Oct, 2015-Jan, 2018.

APPJ experimental set up: The experimental set up of the argon APPJ system is clearly shown in Fig. 1. The jet was generated by blowing argon gas through a capillary metallic tube of 1 mm inner diameter, which is inserted in alumina tube of 3 mm inner diameter, when powered by 8-25 kHz sinusoidal voltage waveform. A grounded copper sheet, of 8 mm width, surrounds the alumina tube above the jet nozzle by 15 mm. The generated plasma jet was characterized electrically, spectroscopically and photographically using two current probes, one voltage probe, 0.5 m spectrometer (Acton SP-2356 imaging spectrograph) with 1800 g mm^{-1} grating and a CCD. The spectra were collected with a Single-leg-fiber optic bundle (LG-455-020-3) camera which is coupled to the spectrometer. The APPJ gas temperature was considered as the rotational temperature of (0,0) band of the second positive of the N_2 system. The gas temperature was estimated by comparing the matched measured and simulated spectra of the nitrogen second positive system (C3P u-B3P g), which is collected at 2 mm below the jet nozzle. Then, the temperature was obtained by best matching of the two spectra as explained previously[23].

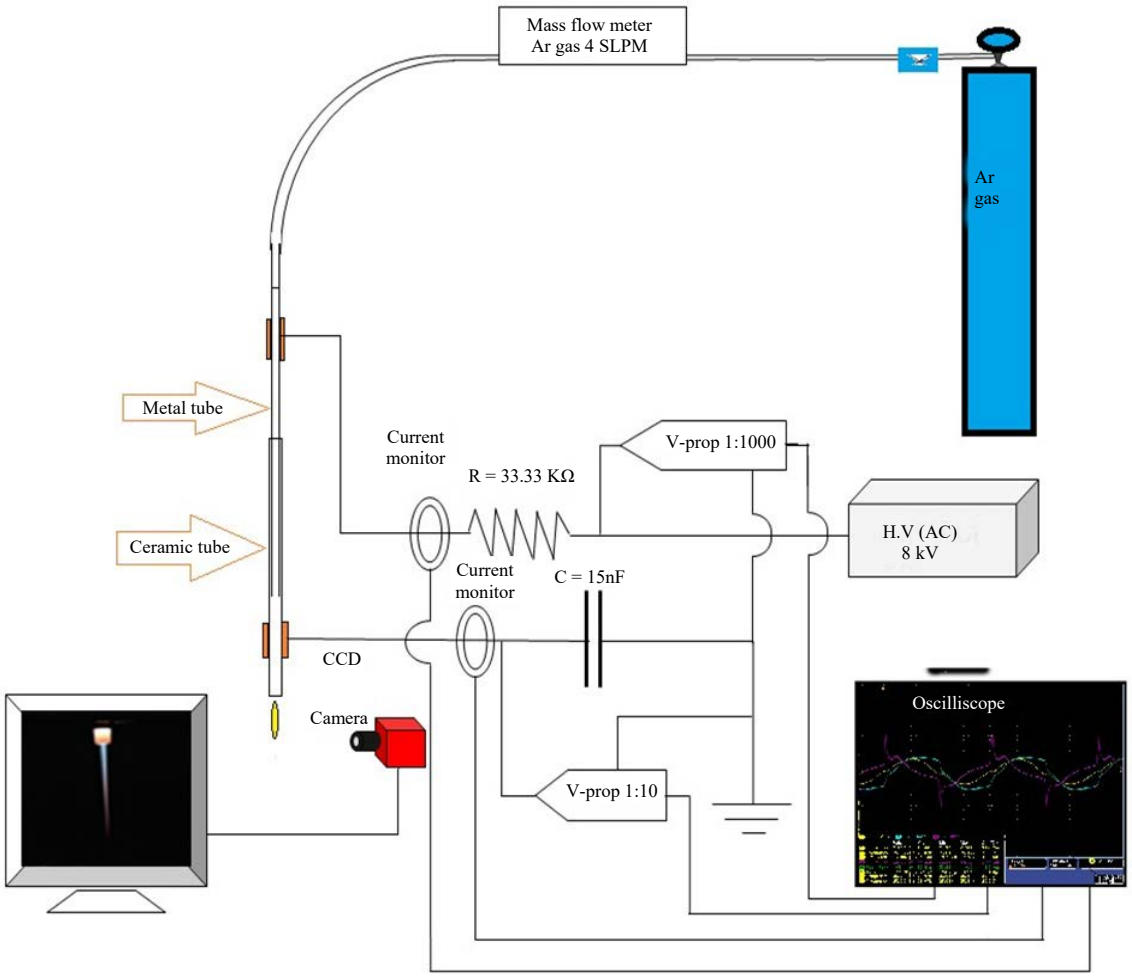

Fig. 1: Schematic diagram of APPJ experiment set up

Microbiological media: For cultivation and subculture of bacteria three types of media were used; first, nutrient agar medium (OXOID, England) with the following composition (g L^{-1}): Yeast extract 2.0, peptone 5.0, sodium chloride 5.0 and Agar 15. This medium was used for preparation of bacterial subcultures. Second, as a liquid medium, LB Broth medium (Bio World, US/Canada) was used in preparation of bacterial suspensions during exposure experiments. Third, for determination of bacterial count, standard plate count medium (OXOID, England) was used. Preparation of media was carried out according to instructions of Manufacturer Company. On the other hand, ready to prepare Sabouraud dextrose agar medium (OXOID, England) was used for cultivation and subculture of fungal culture.

Microorganisms: Two bacterial candidates and one fungal strain namely; *E. coli*, *S. aureus* and *Candida albicans* were used in this study. All strains obtained from the "College of Medicine" in Imam Abdulrahman Bin Faisal University, Saudi Arabia. The strains stored in a refrigerator and regularly subcultured and maintained on nutrient agar for *E. coli* and *S. aureus*, as well as Sabouraud dextrose media *Candida albicans*, respectively.

Plasma exposure: Before exposure, an overnight culture was used in preparation of bacterial or fungal suspension in sterile distilled water or LB broth medium. During the direct exposure, 100-200 µL of the suspension in 0.2 mL Eppendorf tube was placed approximately 10 mm below the jet nozzle. The plasma exposure time interval was ranging from 10-720 s, according to the type and density of microbial cells that ranged from 4.3×10^6 to 1.7×10^7 CFU mL^{-1} for *E. coli* and *S. aureus*, as well as 2.7×10^6 CFU mL^{-1} for *Candida albicans*.

Immediately after plasma exposure, the viable cell count was quantitatively estimated using standard plate count technique[24]. In this method, the samples were taken (in duplicate) from the plasma treated suspension, further diluted, plated on standard plate count medium or Sabouraud dextrose medium. Then the samples were incubated at 37°C for 24 and 25°C to 73 h for bacterial and fungal cultures, respectively.

Statistical analysis: The D-value (decimal reduction time); the time required to kill log10 cells of the exposed organism, was calculated using Excel software program.

RESULTS

APPJ characteristics

Diagnostics of cold plasma jet: The average voltage-current waveforms at 4 SLPM (Standard liter per minute) argon flow rate and 8 kV (peak-to-peak) applied sinusoidal AC voltage at 25 kHz are presented in Fig. 2a. One current peak per each voltage cycle was obtained which is a typical homogeneous dielectric barrier discharge behavior. The current peaks result from the fast increase in the conductivity at plasma ignition followed by an accumulation of surface charge which acts to reduce the applied voltage and extinguish the discharge. The consumed power of the used argon APPJ was calculated using the charge-voltage curve (Lissajous Fig. 2b) to calculate the consumed energy Eel and then it was multiplied by the frequency to get the total consumed power P as shown in Fig. 2b. The consumed power for the operating conditions above (Fig. 2a) was calculated to be 42 W.

Argon flow rate and plasma jet length: Effect of argon flow rate on plasma jet length clearly show that the plasma jet length increases with argon flow rate to reach about 15 mm, as a maximum length, at 4 SLPM. Then, the jet length decreases with increasing the argon flow rate to reach about 4 mm at 9 SLPM. The shrink in plasma jet length is due to the transition from laminar to turbulent flow mode. However, the plasma jet length increases with applied voltage at 4 SLPM constant flow rate. Therefore, 4 SLPM, at which the plasma jet has its longest length, was chosen to be the operating flow for microorganisms' plasma exposure (Fig. 2a-b).

Spectroscopic characteristics: The APPJ emission spectra in the range of 300-400 nm and from 650-850 nm are presented in Fig. 3. The spectra were measured at operating condition of 4 SLPM, 25 kHz and 8 kV at 2 mm below the jet nozzle. The emission spectra clearly shown the presence of OH at 308 nm and O radical at 777 and 844 nm. Moreover, the second positive system of nitrogen molecules, with 0-0 transition at 337.2 nm, was presented strongly in the emission spectra (Fig. 3).

Plasma argon condition: The gas temperature at 2 mm below the jet nozzle and operating condition of 25 kHz and 8 kV was estimated by comparing the emission spectra from the 0-0 transition of the second positive system experimentally and simulation which is presented in Fig. 4 at operating condition of 4 SLPM and 25 kHz. The simulated data was shifted up to clarify the match with the experimental spectrum. The estimated gas temperature was 320±10 K which indicated that the generated plasma is non-thermal plasma (Fig. 4).

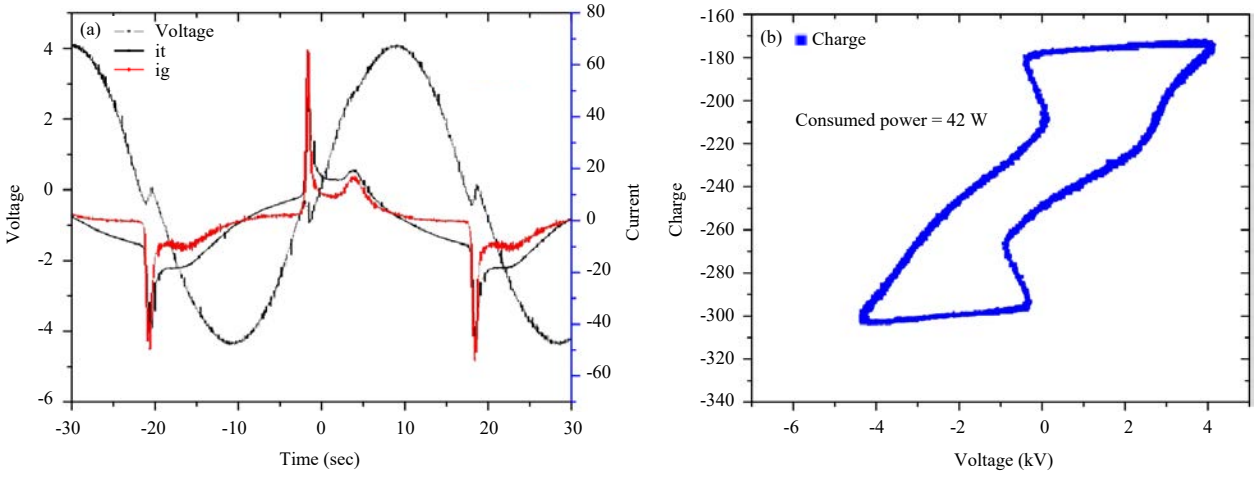

Fig. 2(a-b): (a) APPJ voltage and current waveforms and (b) Power consumption measurements using charge-voltage curve (Lissajous figure) at operating condition of 4 SLPM and 25 kHz

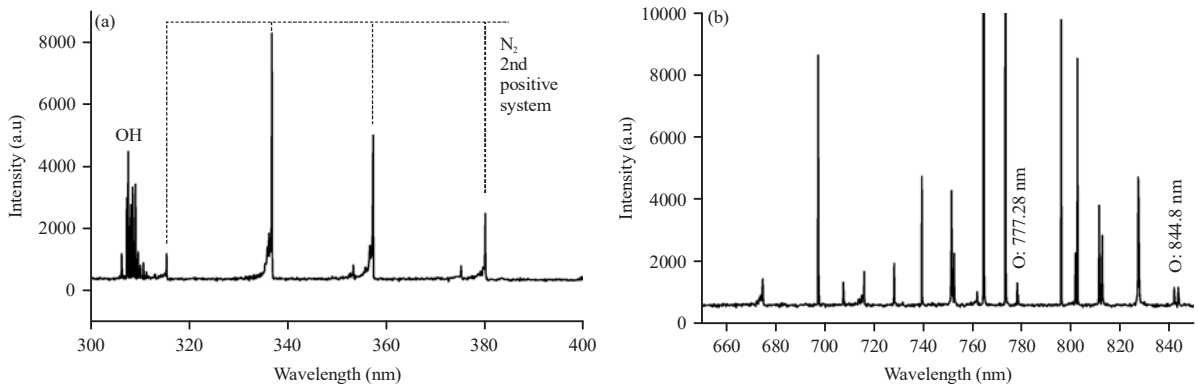

Fig. 3(a-b): Argon APPJ emission spectra at operational condition of 8 kV, 25 kHz and 4 SLPM

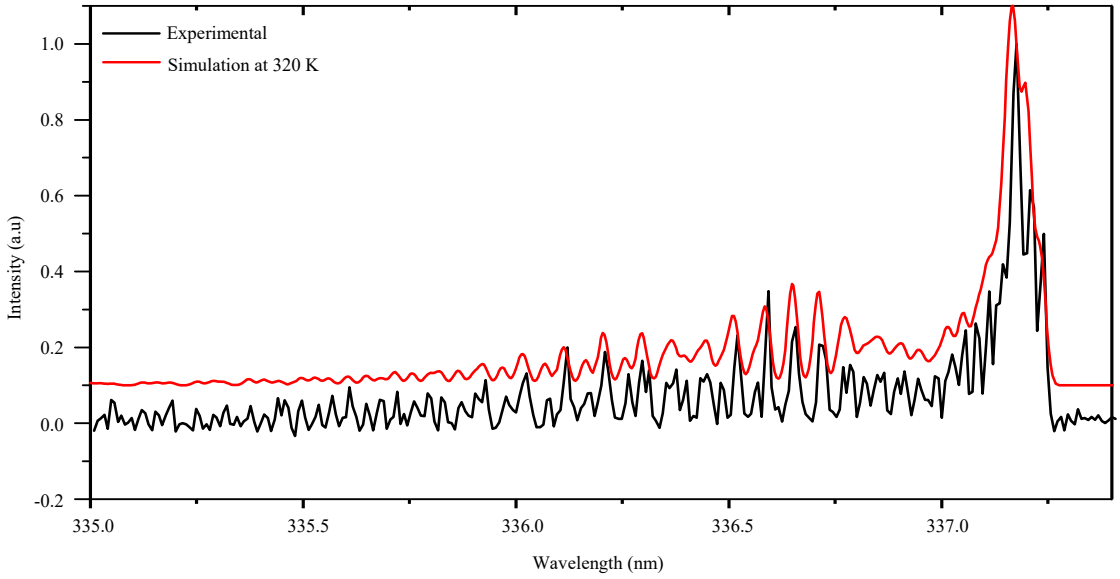

Fig. 4: Argon APPJ rotational temperature estimation at operational condition of 8 kV, 25 kHz and 4 SLPM compared with the simulation spectra of the 2nd positive system of the nitrogen molecules at 320 K

Exposure of microbial cells to argon APPJ plasma

Exposure of *S. aureus* and *E. coli* to APPJ: Results illustrated in Fig. 5 revealed that argon APPJ was adversely affected bacterial cells of both Gram positive and Gram negative bacterial candidates *S. aureus* and *E. coli*, respectively with maximum killing activity after 120-300 s of exposure. After 30 s exposure times to APPJ, reduction in *E. coli* count was approximately 98.3 and 36.1% for cells suspended in water and LB medium, respectively. For *S. aureus*, the reduction was 94.1 and 46.8% for water and LB suspensions, respectively after 30 s APPJ exposure time. As compared with LB complex suspension medium, cells of *E. coli* and *S. aureus* suspended in water showed 2.7- and 2-folds increase in plasma lethal activity after 30 s of exposure, respectively.

Exposure medium and D-value: The influence of exposure media namely; water or LB medium, on the lethal effect of plasma was confirmed by the results of D-value (decimal reduction time) illustrated in Table 1. In presence of water medium, the D-values for *E. coli* and *S. aureus* recorded 37 and 34 s, respectively. This effect was significantly reduced in presence of LB medium and recorded 333 and 476 s, for *E. coli* and *S. aureus*, respectively.

Exposure of *Candida albicans* to APPJ: Results graphically illustrated in Fig. 6 revealed that the plasma irradiation adversely affected unicellular pathogenic fungus *Candida albicans* cells count with approximately 71.8% reduction in living cell count after the 20 s. In presence of LB complex medium the lethal activity reduced by 1.2 fold due to

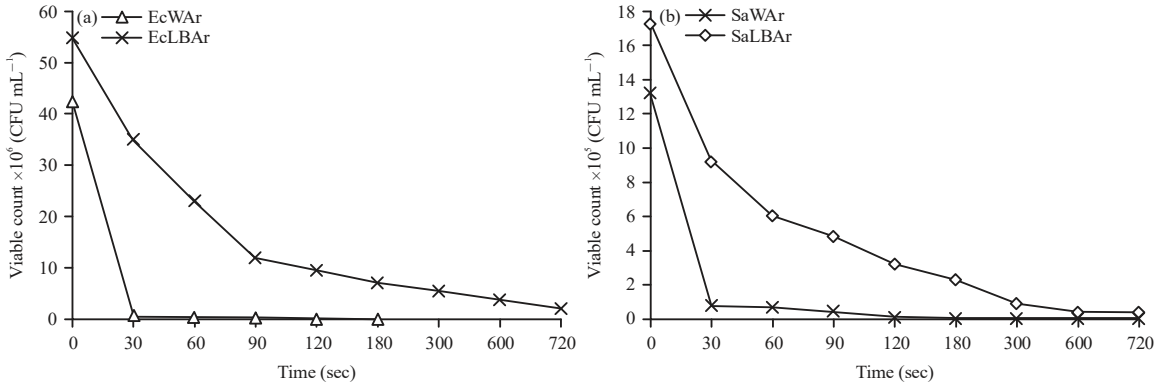

Fig. 5: Reduction in (a) *E. coli* and (b) *S. aureus* bacterial cell count due to argon APPJ for different exposure time intervals

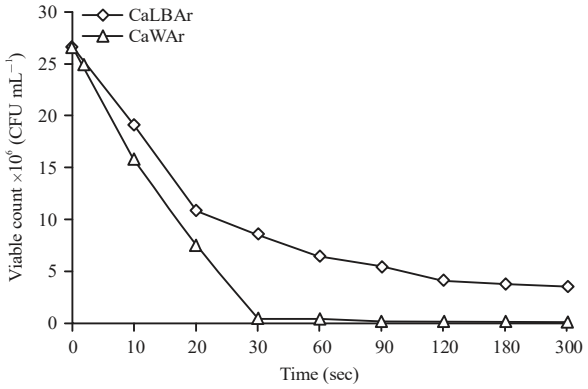

Fig. 6: Living cell count of *Candida albicans* cells suspended in water and in LB medium after exposure to argon plasma for different time intervals

Table 1: D-values for various APPJ decontamination conditions

Microorganism	Media	D-value (s)
S. aureus	LB	476
S. aureus	Water	34
E. coli	LB	333
E. coli	Water	37
C. albicans	LB	385
C. albicans	Water	139

the delayed antimicrobial effect of argon plasma (Fig. 6). This assumption was also confirmed by the results reported in Table 1, where the D-values was increased from 139-385 s due to the influence of organic matter content of the medium that hinders the activity of many plasma species.

DISCUSSION

In this study, an experimental argon APPJ was designed and the antimicrobial effect of generated plasma on a group of pathogenic bacterial and fungal candidates was determined. Results indicated that the plasma showed characteristic homogeneous dielectric barrier discharge behavior[25]. The emission spectra show the presence of the reactive species such as OH and O which have a great contribution to the microbial inactivation[26]. At atmospheric pressure the collision frequency between plasma contents is very high. Therefore, the energy transfers from the translational energy to the rotational energy are very fast 10-6 s[27]. As a result, the rotational temperature is considered to be as the gas temperature. The results in Fig. 4 clearly shown that the gas temperature is in the range of 320 K which is a clear indication that the generated plasma is a non-thermal plasma. Thus, the generated plasma can be used for the treatment of temperature sensitive materials.

Also, emission spectra of the generated APPJ plasma was able to induce high levels of reactive oxygen species e.g., H_2O_2, •OH, •O, $•O_2^-$ radicals, as well as second positive system of nitrogen molecules. Singh *et al.*[28] reported that plasma dissociates the atmospheric O_2 and N_2 and leads to the formation of NO_2^- and NO_3^-, thus can be used in disinfection of water. Generally, the reactive free radicals (ROS and RNS) generated by plasma are responsible for oxidative damage of many cell targets namely DNA, RNA, proteins and lipids. Lipid peroxidation due to •OH, protein aggregation through interaction with thiol groups and physical destruction of DNA oxidative are the major consequences of such damage. Moreover, most of lipid bilayer membrane receptors and proteins are interrupted and cytosolic cell contents are released due to disruption by ROS. These findings are in concordance with many results obtained by several scientists Shimizu *et al.*[6], Nosenko *et al.*[7], Pignata *et al.*[14], Deng *et al.*[29], Burts *et al.*[30], Yang *et al.*[31] and Von Keudell *et al.*[32]. In the present study, the reduction of cell count due to argon cold plasma, for *E. coli* and *S. aureus*, was significant.

Colagar *et al.*[13], Burts *et al.*[30] and Abreu *et al.*[33] recorded that plasma generated from an argon or other gases might lead to decrease in living cell counts for many bacterial species. Interestingly, *E. coli* was completely disinfected after 120-180s exposure times. In concordance with the current results, Colagar *et al.*[13] reported that *E. coli* disinfected 100% after 300s. Results also indicated that microbial load may affect antimicrobial activity in some cases, as previously described by Fernández and Thompson[10] and Fernández *et al.*[34]. Generally, LB medium does not completely interfere with plasma irradiation and the cell mortality remains, the delayed effect was only recognized in some cases. D-values were increased from 34, 37 and 139-333s, 476 and 385s for *E. coli*, *S. aureus* and *C. albicans*, respectively, due to delay in plasma destructive action. A similar effect was reported by Nosenko *et al.*[7], they mentioned that LB and PBS media have no effect on cell mortality. In accordance, Nosenko *et al.*[7] and Pollak *et al.*[35], suggested that the presence of bacteria in the form of a suspension in LB nutritive complex medium reduces the efficiency of plasma and UV irradiation. This is due to geometry of cells distribution in the complex medium (shadowing effect) as well as buffering (antioxidative) activity of the microbial cells in the suspension.

Interestingly, the disinfection of *Candida albicans* using plasma generated from Argon is recorded as a promising result. Similarly, Klämpfl *et al.*[11] reported the possible use of air plasma for the disinfection of surfaces occupied with Gram positive and Gram negative bacteria, bacteria endospores and *Candida albicans*. The effect of He/O_2 plasma on *Candida albicans* biofilm was also reported by Sun *et al.*[36]. They found that plasma generated by the designed system, with or without the drug, has an antifungal effect on the yeast-like fungi. Additionally, the generated reactive oxygen species (ROS) are the possible mechanism for the germicidal activity. ROS was proven to cause damage to DNA and cytoplasmic membranes by combination with DNA as well as oxidative damage of membrane lipids and proteins which led to impairment of some vital elements transition[36]. The results collectively could provide a basis for developing novel approaches in treating many diseases caused by pathogenic candidates belonging to *E. coli*, *S. aureus* and *C. albicans*.

CONCLUSION

Generated argon cold APPJ plasma is able to induce ROS with lethal effect on some microbial candidates, thus can be considered as a promising technology for disinfection of surfaces occupied microbes by genera belong to *E. coli*, *S. aureus* and *C. albicans*. The destructive action of plasma was greatly affected by exposure time, shadowing and buffering effect of complex organic substances that could be present in the medium. The current challenge is to increase penetration power of plasma and optimize media for progressive generation of reactive free radicals (ROS and RNS).

SIGNIFICANCE STATEMENT

This study revealed that the argon cold APPJ plasma can be a promising technique in disinfections of surfaces occupied with microorganisms. Indeed, the study will help researchers to test the possible use of this atmospheric pressure plasma jet (APPJ) approach for treatment of many diseases, especially caused by antibiotic-resistant pathogens.

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