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### Abstract

Oxygen activated cold-atmospheric-pressure-argon plasma jet (APPJ) has gained prominence over the regular argon plasma especially in disinfection and decontamination. As an objective of the current research, an oxygen-enriched argon system was built, where plasma produced through a vessel metallic tube that is introduced into alumina one. A sinusoidal high voltage signal of 25 kHz was used to generate plasma jet. Potential impact of oxygen enriched APP jet (Ar/O<sub>2</sub>) in decontamination of different microbial cells was observed. For examination, suspension of each tested microbe was placed in contact with plasma jet nearly 10 mm away from the jet nozzle and the lethal effect of activated plasma was evaluated. Results showed that the Ar/O<sub>2</sub>-APP plasma enhanced reactive hydroxyl and other reactive oxygen species generation and hence, antimicrobial activities. For *E. coli* cells approximately 96.27% and 90.50% reduction in living cell count were recorded after 30 s of exposure to oxygen-activated plasma, on water and LB medium, with a D-value of 36.21 s and 588.24 s, respectively. Also, reduction in *S. aureus* cells was 92.8% and 96.6% after 30 s, with the D-values of 33.9 s and 181.8 s in water and LB media, respectively. Moreover, *C. albicans* fungal cells revealed 95% reduction in viable cell count after 10 s. However, the D-value showed only 6.3 s increase due to the impact of organic materials as a component of LB nutritive medium. Generally, the activated argon plasma works effectively even in the presence of organic debris in an exposed medium. Therefore, APPJ can be considered as a prominent straightforward technology for medication of diseases caused by antimicrobial drug-resistant pathogens.

### Keywords

Oxygen-argon plasma, Ar/O<sub>2</sub> plasma, antimicrobial resistance, *E. coli*, *C. albicans*, *S. aureus*, Cold plasma

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## RESEARCH PAPER

# Potential Enhancement of Microbial Disinfection Using Oxygen Enriched Cold Atmospheric-pressure Argon (Ar/O<sub>2</sub>) Plasma Jet

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## Abstract

Oxygen activated cold-atmospheric-pressure-argon plasma jet (APPJ) has gained prominence over the regular argon plasma especially in disinfection and decontamination. As an objective of the current research, an oxygen-enriched argon system was built, where plasma produced through a vessel metallic tube that is introduced into alumina one. A sinusoidal high voltage signal of 25 kHz was used to generate plasma jet. Potential impact of oxygen enriched APP jet (Ar/O<sub>2</sub>) in decontamination of different microbial cells was observed. For examination, suspension of each tested microbe was placed in contact with plasma jet nearly 10 mm away from the jet nozzle and the lethal effect of activated plasma was evaluated. Results showed that the Ar/O<sub>2</sub>-APP plasma enhanced reactive hydroxyl and other reactive oxygen species generation and hence, antimicrobial activities. For *Escherichia coli* cells approximately 96.27% and 90.50% reduction in living cell count were recorded after 30 s of exposure to oxygen-activated plasma, on water and LB medium, with a D-value of 36.21 s and 588.24 s, respectively. Also, reduction in *Staphylococcus aureus* cells was 92.8% and 96.6% after 30 s, with the D-values of 33.9 s and 181.8 s in water and LB media, respectively. Moreover, *Candida albicans* fungal cells revealed 95% reduction in viable cell count after 10 s. However, the D-value showed only 6.3 s increase due to the impact of organic materials as a component of LB nutritive medium. Generally, the activated argon plasma works effectively even in the presence of organic debris in an exposed medium. Therefore, APPJ can be considered as a prominent straightforward technology for medication of diseases caused by antimicrobial drug-resistant pathogens.

**Keywords:** Oxygen-argon plasma, Ar/O<sub>2</sub> plasma, Antimicrobial resistance, *E. coli*, *C. albicans*, *S. aureus*, Cold plasma

## 1. Introduction

Atmospheric-pressure cold plasma technology has been entering a new era as an efficient cutting-edge technology in a wide range of applications in medicine, food industry, and environment such as sterilization, disinfection, food

preservation, improvement of crop yield, and inactivation of toxins [1–3]. The advantage of the cold plasma technology comes from its non-thermal property, generation under conventional atmospheric pressure, low cost, simplicity, ease of use, non-toxicity, and nearly without side effects [4–8].

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The main reason for use of cold atmospheric-pressure plasma technology in disinfection and sterilization is the generation of many excited molecules and atoms including reactive oxygen (ROS) and nitrogen species (RNS) e.g.,  $O_3$ ,  $H_2O_2$ , OH,  $O^{2-}$ , NO and  $NO_2$  that have counteracting lethal effects on many microbial and mammals' systems [9].

Many researchers have been working on applying cold plasma in decontamination and disinfection. The role of plasma in inactivation of *Pseudomonas aeruginosa* biofilms has been described by Vandervoort and Brelles-Marino [10]. Gupta and Ayan [11] reviewed the detailed mechanism of argon plasma in inactivation of nosocomial biofilm formation by several pathogenic microbes. A few systems have been utilized to overcome the confinements to produce cold atmospheric-pressure plasma in air such as segmented cathode [12].

Atmospheric-pressure plasma jet (APPJ) is an accessible plasma since it can be produced with no need for any vacuum equipment or sophisticated materials. Furthermore, it is a versatile system consisting of a pen-like shape connected to high voltage power supply and a gas source. This simple construction makes it possible to direct the system easily into objects and biological samples [13]. Also, the APPJs can be applied under different operation conditions to fit diverse targets and applications [14–16].

Because of its minimal breakdown voltage and gas temperature, helium plasma jet is highly stable and straightforward technology for creation of APPJ. However, the cost of helium limits its economic use. Compared to argon, air and nitrogen have larger breakdown voltages. Therefore, argon is an appropriate gas that can replace helium in industrial and medical applications. Thus, a special interest has been given to argon gas as source of plasma instead of helium, nitrogen, or air because it is cheaper and has a low breakdown voltage [17]. Interestingly, utilizing argon increases the APPJ gas temperature and improves the probability of glow-to-arc transition. Though, these argon plasmas have low power consumption, inexpensive, and have high sterilization efficacy [18]. Interestingly, the use of activated argon plasma has gained a wide interest. Choe et al. [19] explored the enhancements of argon-based plasma characteristics by mixing it with He and  $O_2$ . It was concluded that the newly generated plasma improved industrial applications of atmospheric plasma. Wang et al. [20] revealed that the oxygen-argon plasma exhibits unique diagnostic characteristics that are different from He/ $O_2$  plasma. The antimicrobial effect of argon/oxygen

(Ar/ $O_2$ ) cold plasma against *Enterococcus faecalis* biofilm was more efficient than other chemical disinfectants within a time of 20 min [21]. When the input gas is combined with additional gases, the reactive species (OH, NO, and O) produced by the plasma jet can be enhanced. Therefore, to attain the optimal level of O-radical generation, oxygen gas is injected in different ratios [22]. All plasma generations, even in a basic gas mixture, become somewhat complex due to the presence of oxygen [23]. Reineke et al. [24] argued the role of different plasma types in the activation of *Bacillus* spores. They found that oxygen-argon plasma generates 4 times UV photons of more reactive oxygen and nitrogen species than for pure argon and to be more effective in inactivation process. Also, cold argon and oxygen activated ones have been applied in the degradation of toxic chemical perchloroethylene (PCE) [25].

Recently, development of antibiotic and drug resistance among microbial pathogens are rapidly escalating and becoming a crucial worldwide health problem. This will certainly results in increased patient morbidity, mortality, hospital stays and cost. Therefore, it is an essential element of demand to find an innovative method to overcome this problem [26,27]. Especially for getting rid of several microbial infections that have been connected to blood, urinary tract infections, endocarditis, gastroenteritis, organ malfunction, or soft tissue infections [28].

One of the suggested techniques to inhibit microbes especially bacteria such as *Staphylococcus aureus* and *Escherichia coli* is to apply tap water treated by plasma in medicine (plasma-activated water; PAW) using gliding arc [29,30]. Also, Khosravi et al. exposed *E. coli* and *S. aureus* biofilms to an atmospheric-pressure dielectric-barrier discharge (DBD) plasma [31]. Moreover, Zhang et al. used air plasma jet to inactivate bacteria (*E. coli* and *S. aureus*) and fungi (*T. rubrum* and *Candida albicans*) [32].

The main aim of the current study is to investigate the use of argon/oxygen (Ar/ $O_2$ ) atmospheric-pressure activated plasma jet as a potential approach for microbial disinfection. Therefore, the objective of the current research is to design an easy system of argon/oxygen (Ar/ $O_2$ ) atmospheric-pressure activated plasma jet and further characterize the generated plasma. Subsequently, assess the potential lethal effect of generated plasma on unicellular microbial cells such as *E. coli*, *C. albicans*, and *S. aureus*. Moreover, emphasis was given to the contribution of medium composition on the potential disinfection power of the oxygen enriched argon plasma during exposure process.

## 2. Methods and materials

### 2.1. Cold atmospheric-pressure plasma jet

The plasma jet system consists of a tube of alumina ceramic insulator with a length of 150 mm that has inner and external diameters of 3 and 4.8 mm, respectively. The quality of the alumina tube is 99.7%. Two identical copper rings with a width of 6 mm and thickness of 0.48 mm surround the alumina tube. The distance between the two rings is 10.2 mm. The lower ring is 5 mm away from the tube's nozzle and is connected to the ground. The upper ring serves as a high-voltage electrode that is connected to a sinusoidal AC-high voltage signal. The electrical characteristics of the PLASMA JET were recorded using a 1 GHz-5 GS/s digital phosphor oscilloscope (Tektronix DPO 4104B) with a high-voltage probe (Tektronix P6015) and two Pearson current monitor probes (model 6585). A plasma jet is produced in the alumina tube by the flow of 99.999% pure argon and oxygen gases when an AC voltage is applied between the two electrodes. Mass flow meters (Alicat Scientific MC-5SLPM-D and MC-20SLPM-D) are used to control and monitor the gases flow (Fig. 1).

An image spectrograph (Acton SP-2356), which is a triple grating with a 500 mm focal length, optical emission spectroscopy was used to characterize the emission spectra of the generated plasma jet in air. First grating: 3600 gr/mm is sensitive between 200 and 450 nm and is blazed at 253.65 nm. Conversely, the second grating has a blaze at 500 nm. It is 1800 gr/mm and sensitive between 450 and 850 nm. Two gratings were applied through this work. The spectrograph is equipped with an integrated high-sensitivity photomultiplier detector (type ARC-P2,

Princeton instrument) with a sensitivity range of 190–900 nm, and it is connected to a single-leg fiber optic bundle (LG-455-020-3). The resolution of the optical emission spectroscopy can reach 0.02 nm depending on the grating and slit width used in the experiment.

At atmospheric pressure, the frequency of collisions between the constituents of plasma is relatively high. Consequently, there is a rapid energy transfer from the translational energy to the rotational energy,  $10^{-6}$  s [33,34]. Consequently, the rotational temperature can be considered as the gas temperature. As a result, the plasma jet gas temperature was calculated by evaluating the rotational (0,0) band of the second positive  $N_2$  system, second positive system  $N_2$  ( $C^3\Pi_u - B^3\Pi_g$ ), ( $C^3\Pi_u, v' = 0 \rightarrow B^3\Pi_g, v'' = 0$ ). The experimentally measured spectrum was compared to a stimulated spectrum. Our group imported the gas temperature code J. K Lee group in Korea [17]. The estimated gas temperature was measured at 2 mm below the jet nozzle.

### 2.2. Microbial cells and cultivation media

To test the potential disinfection capability of oxygen-activated argon plasma, a group of bacterial and fungal candidates namely, *S. aureus*, *E. coli*, and *C. albicans* were used. Those candidates brought from the culture collection, College of Medicine, Imam Abdulrahman Bin Faisal University (IAU), Dammam, Saudi Arabia. The bacterial and fungal cells' cultivation, subculture, and subsequent living cell counts after exposure to activated argon, were carried out as previously described by Younis et al. [35]. It is evident that the created plasma jet is a non-thermal plasma because

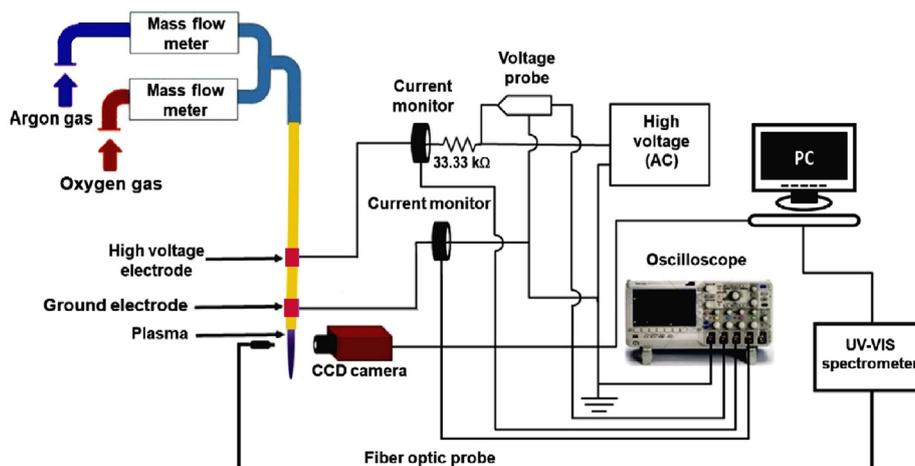


Fig. 1. Plasma jet's experimental setup.

its estimated gas temperature was in the region of 320 K. Thus, the generated plasma jet can be used to treat materials that are sensitive to temperature.

### 2.3. Exposure of the investigated bacteria to oxygen-activated argon plasma

For exposure studies, microbial cells from 24-h preculture were suspended in sterile H<sub>2</sub>O or LB medium. The typical composition of the medium “LB medium” or “Luria Bertani” was: tryptophane, 10 g/L; yeast extract, 5 g/L; NaCl, 10 g/L. This medium was prepared and sterilized according to the manufacturer's instructions. Activated argon plasma jet was positioned 10 mm from the surface of approximately 100–200  $\mu$ l of cell suspension in 0.2 mL Eppendorf for 10–720 s during the exposure processes. This was done in accordance with the type and concentration of microbial cells, that varied from  $2.7 \times 10^6$  CFU/mL for *C. albicans* to  $4.3 \times 10^6$  to  $1.7 \times 10^7$  CFU/mL for *E. coli* and *S. aureus*. The living cell count was determined to study the influence of plasma on the microbial cells. To test the lethal effect of activated argon plasma on the cell's suspensions, samples were taken after exposure. Subsequently, the samples were diluted and sub-culture on nutritive media (standard plate count for bacteria and Sabouraud dextrose for unicellular fungi) as previously reported [36]. Finally, plates were incubated at 37 °C for 24 h and 25 °C for 73 h for bacterial and fungal cultures, respectively. For accuracy, samples were taken in duplicate.

### 2.4. Statistical analysis

The D-value was calculated using an excel software program to determine the time needed to kill  $\log_{10}$  cells of the exposed organism.

## 3. Results and discussion

### 3.1. Oxygen gas mixture effect

Fig. 2 shows the current versus–voltage for the generated plasma with pure argon (a) and up to 4 % mixed oxygen (0.018/4.5 slpm) (b) at an applied voltage of 14 kV and a frequency of 30 kHz.

In this study, the values of current represent the peak values. When mixing oxygen to argon gas, the peak of current to ground increases from about 8.3 mA to about 22.9 mA that is somewhat higher than peak of the total current during the positive half cycle. However, the total current peak rises from about 11.3 mA to about 18 mA. During the negative half cycle, the peak of total current

increases from around 11.6 mA to around 17.2 mA. The ground current grows from nearly 8.1 mA to nearly 16.6 mA. In addition, when the applied voltage increases, there are more current peaks for each half cycle [37]. However, when oxygen is added to argon, the current–voltage waveform displays a similar behavior, with an increase in the number of current peaks each half cycle. This may be due to an increase in the oxygen electron affinity-related number density of the residual charge carriers from the prior pulse [22].

The appearance of a one single sharp pulse in the total current waveform per each half a cycle of the applied voltage elucidates the formation of a homogeneous discharge [38]. Also, some small spikes observed on the current peak due to the formation of some streamer in addition to the homogeneous discharge. The generation of discharge current pulses every half cycle is associated with the applied voltage. As the voltage increases, the high external electric field increases and a process of charges accumulation on the dielectric tube starts until the internal field reaches the breakdown point. Therefore, the first current pulse is formed. Once the first pulse is formed, a process of depletion of the accumulated charges begins [39]. However, a further increase in the voltage or mixing oxygen to the operating gas results in the formation of a new accumulation of charges. Then, a second discharge current pulse is ignited [40]. The lifetime of the residual charges generated during the first pulse accelerates and facilitates the ignition of the second pulse [41]. The first current peak is more sharper than the second current pulse owing to the number of accumulated charges [39]. More details on the current voltage wave from various mechanisms have been previously published [33,42–45].

According to plasma jet imaging, compared to pure argon, mixing oxygen to the plasma jet speeds up the transition from filamentary to diffused mode at lower values of flow rate (two examples of images for plasma plume for two different percentages of mixing oxygen to argon are presented in Fig. 3). Typically, the filament is generated within the diffused plasma at applied voltage, flow rate, and frequency of 14 kV, 4.5 slpm, and 30 kHz, respectively [45]. By introducing oxygen, the filament disappears and the length of plasma plume shrinks. In addition, the gas temperature increases with the addition of oxygen to argon plasma jet.

The height of peaks in the given examples of the emission spectra displayed in Fig. 4 demonstrates that the intensity of the plasma plume reduces with the rise of oxygen percentage.

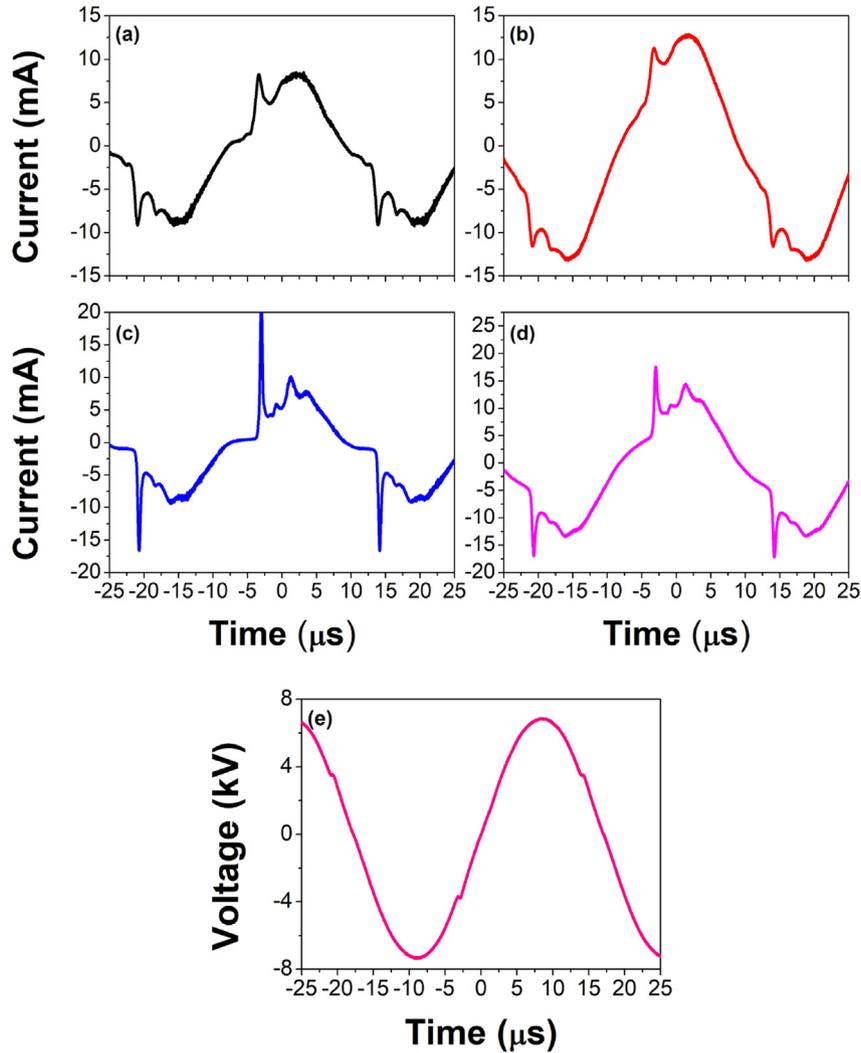


Fig. 2. Waveforms of (a) ground current and (b) total current in pure argon and waveforms of (c) ground and (d) total current for argon mixed with (0.018/4.5) slpm for oxygen/argon flow rates, and (e) the applied voltage. The frequency and the value of the applied voltages are 30 kHz and 14 kV, respectively.

| O <sub>2</sub> mixed (%) | 0.0   | 0.6   | 1.2   |
|--------------------------|-------|-------|-------|
| PLASMA JET image         |       |       |       |
|                          | 320 K | 330 K | 380 K |

Fig. 3. Images and values of estimated temperature of plasma jet plasma plume for pure-argon and two different percentages of mixing oxygen to argon at operating conditions of an applied voltage of 14 kV, a frequency of 30 kHz, and a flow rate of 4.5 slpm.

Fig. 5 shows the oxygen and OH radicals against the oxygen percentage. It implies that the intensity of oxygen lines intensity increases by progressive increase in oxygen supply till reaching its maximum peak at approximately 0.8% and then decline further with the increase in oxygen percentage. The OH-radicals intensity decreases with increasing the percentage of oxygen addition. Moreover, with only a little amount of oxygen addition, factors such as the electron energy losses, the number of excitations and ionizations, and dissociation processes work together to reduce electron temperature [23]. However, a variety of positive and negative ions (O<sup>+</sup>, O<sub>2</sub><sup>+</sup>, O<sub>4</sub><sup>+</sup>, O<sub>3</sub><sup>-</sup>, O<sub>2</sub><sup>-</sup>, O<sup>-</sup>) are effectively produced when electrons collide with oxygen [23]. The intensity of all these radicals decreases with increasing the percentage of oxygen addition as illustrated in

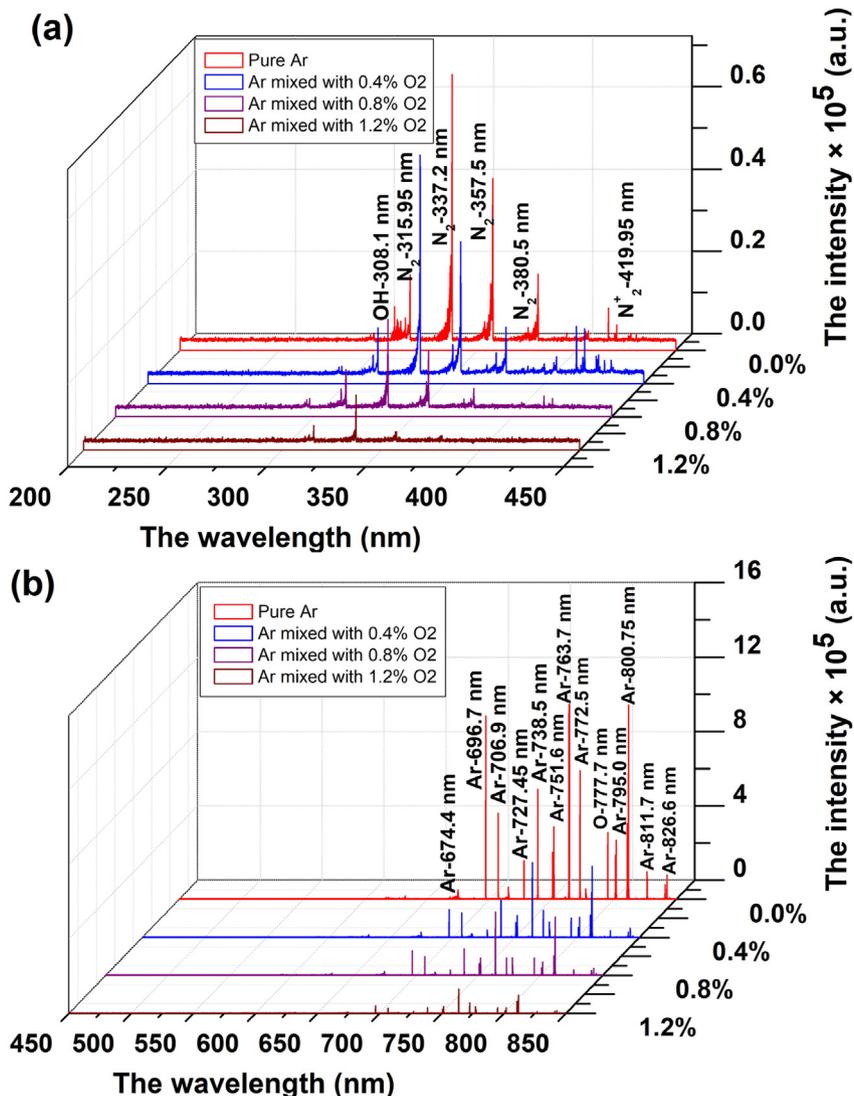


Fig. 4. Optical emission spectra of the plasma jet in the wavelength ranges (a) 200–450 nm and (b) 450–850 nm for pure argon (4.5 slpm) and various oxygen concentrations at 14 kV and 30 kHz.

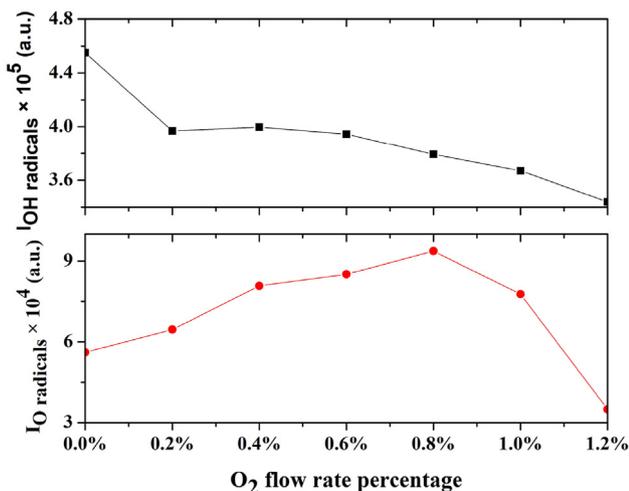


Fig. 5. Oxygen and OH radicals versus oxygen concentrations.

Fig. 5. Oxygen is an electronegative gas. Thus, when it is added to the operating argon plasma jet, it rises electron losses, working like scavenger for electrons. Therefore, the electron energy losses, dissociation processes, the number of excitations, and the number of ionizations are influenced by the oxygen mixture [22]. Also, oxygen-related ions can significantly increase the plasma jet gas temperature. Especially, dissociative electron attachment leads to effective  $O^- + O$  that has activated at energies lower than 5 eV [37]. Simultaneously, the dissociative process decreases electron density and reduces electron energy that promotes gas heating [23]. The electronegativity and distinctive characteristic of oxygen is responsible for energy transfer from electrons to an overall rise in gas temperature [23]. Further details on Ar/O<sub>2</sub> plasma reactions and

diffusion of ambient air diffusion to the plasma jet can be found in Refs. [45–49].

### 3.2. Antimicrobial activity of oxygen-activated plasma generated by the plasma jet on gram-positive and gram-negative bacteria either in water or complex organic matter containing media

In this experiment the lethal effect of activated oxygen-argon plasma against two different bacteria belonging to gram negative and positive bacterial candidates namely; *S. aureus* and *E. coli* was investigated. Generally, the *E. coli* bacterium is commonly used as a reference strain in the development of new sterilizing technologies [50]. It causes many diseases such as diarrheal, bacteremia, and abdominal infections. Preliminary studies revealed that the plasma jet for pure argon or argon mixed with oxygen led to drastic decrease on *E. coli* colony-forming units (CFUs) at operating parameters of 14 kV, 30 kHz and 4.5 slpm. All treatments for *E. coli* result in a steady decrease in its count. The maximum reduction is achieved from plasma generated of argon mixed with 0.2% oxygen (data not shown). Results shown in Fig. 6 (upper) indicates that the oxygen-argon plasma generated by the experimental system designed in this study has potential lethal effect on *E. coli* cells in either water or complex organic matter containing media. Only 3.73 % and 9.5 % of *E. coli* cells remain alive, after 30 s of exposure to oxygen-activated plasma, on water and LB medium, respectively. In fact, 180 s were enough to ensure complete destruction of the cells suspended in water, while in LB longer time was needed (more than 720 s). On the other hand, results of the D-value shown in Table 1 indicated that the *E. coli* cells was 36.21 s and 588.24 s for cells suspended in water and LB media, respectively.

Interestingly, several studies revealed that potential of microbial inactivation is affected by many factors including the microbial types, physiological state and numbers, inactivation medium, running gas mixture, and gas flow [51]. Remarkably, the potential attack of bacterial and fungal cell walls as well as their membranes by generated reactive oxygen species with insufficient quick repairs or recovery from damage, may lead to their rupture and consequently cell destruction [52,53]. Also, activated argon plasma may cause destruction of microbial cells structure through cracking, devastation and damage of their cells' wall and membrane, and hence a leakage of cellular components from the cells [54]. On the other hand, in vitro exposure of *Aspergillus flavus* to cold atmospheric argon plasma powered at 40 W for 25 min, led to complete

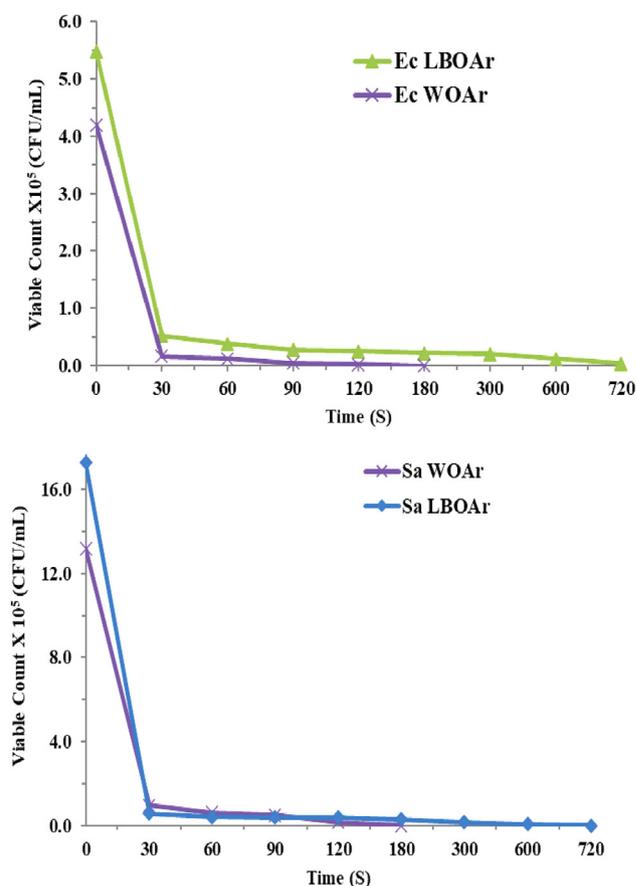


Fig. 6. Reduced *E. coli* (upper) and *S. aureus* (lower) bacterial cell counts because of argon APPJ during various exposure times under an applied voltage of 14 kV, a frequency of 30 kHz, and a flow rate of 4.5 slpm.

Table 1. The D-values of different microorganisms during disinfection with oxygen-argon ( $Ar/O_2$ ) cold atmospheric plasma at operating conditions of an applied voltage of 14 kV, a frequency of 30 kHz, and a flow rate of 4.5 slpm.

| Microorganism      | Media | D-value (s) |
|--------------------|-------|-------------|
| <i>S. aureus</i>   | LB    | 181.82      |
| <i>S. aureus</i>   | Water | 33.9        |
| <i>E. coli</i>     | LB    | 588.24      |
| <i>E. coli</i>     | Water | 36.21       |
| <i>C. albicans</i> | LB    | 128.21      |
| <i>C. albicans</i> | Water | 121.95      |

inhibition of growth, while only 20 min of plasma exposure of brown rice cereal bars led to their protection against *A. flavus* for more than 20 days when stored at 25 °C and full relative humidity [55].

Critzer et al. [56] revealed the capability of activated plasma to ease fresh bacterial biofilms. Fernandez et al. [57] affirmed that cold gas plasma achieved 2.72, 1.76 and 0.94 log-reduction in number of the mostly common enteric pathogen *Salmonella typhimurium* colonizing some vegetables and fruits within 15 min through reactive oxygen species and

electrostatic force. Also, Surowsky et al. [58] found that the microbial load of *Citrobacter freundii* bacterium on apple juice was decreased for up to 5 log cycles due to exposure to argon plasma and 0.1% oxygen for 8 min and subsequent storage for 24 h without need for direct contact. Van Gils et al. [59] used RF argon plasma jet to inactivate *P. aeruginosa* in aqueous medium and showed that reactive oxygen species were the main cause for deactivation. According to Surowsky et al. [58], antioxidants in apple juice may play a crucial role in attenuating reactive species by scavenging them.

Generally, most bacterial candidates belonging to *S. aureus* can cause many inflammatory diseases such as abscesses, endocarditis, pneumonia, and osteomyelitis. Therefore, the antimicrobial activity of the activated argon plasma on the gram positive bacterial candidates namely, *S. aureus* was investigated. Interestingly, the percent reduction in living *S. aureus* cells was 92.8% and 96.6% after 30 s when incubated in water and LB exposure media, respectively, as shown in Fig. 6 (lower). Interestingly, the delay in complete cell destruction between cells incubated in water and LB medium was approximately 600 s. This result was supported by the finding that the D-values for *S. aureus* cells suspended on water and LB media were 33.9 s and 181.8 s, respectively (Table 1).

Results collectively revealed that the oxygen-argon plasma generated from the current experimental system showed characteristic lethal activity against the gram negative bacterium *E. coli* and the gram positive bacterium *S. aureus* in both types of exposure media. Mainly due to creation of efficient excited molecules and atoms including reactive hydroxyl, oxygen, and nitrogen species with potential antimicrobial activities [9,60]. The reactive atoms and ions interact with many cell constituents e.g., cell membrane, nucleic acids DNA and RNA, lipids and proteins leading to cells destruction. Moreover, as compared to a previous study reported by Younis et al. [35], the D-values mentioned in Table 1 indicates that activation of argon with O<sub>2</sub> has more potential antimicrobial activity against the current examined microbes than using pure argon alone under the same investigation conditions.

In concordance with the data generated in the current study, Mortazavi et al. [61] revealed that only 15 min was required to destroy cells in liquid media and only 30 s was enough to get rid of the cells on solid media. Also, enrichment of argon plasma with oxygen showed potential antimicrobial effect against gram positive nosocomial pathogen *Staphylococcus epidermidis* at 0.2% O<sub>2</sub>. With complete

lethal effect after 8 min or at 0.4% O<sub>2</sub> applied to 16 min [62].

In general, the complex LB medium does not completely hinder the generated plasma. Nosenko et al. [63] revealed that complex media doesn't affect cell mortality. Other studies show that the use of LB complex medium led to lower the efficiency of plasma irradiation and delay in its destructive action on bacteria. Moreover, Nosenko et al. [63] and Polak et al. [64] proposed that LB nutritive complex medium or presence of bacterial in aqueous medium reduce the efficacy of plasma and UV treatment by shadowing and buffering antioxidative activity of the bacterial suspension.

### 3.3. Antimicrobial activity of oxygen-activated plasma generated by the plasma jet on *C. albicans* either in water or in complex organic matter containing media.

*C. albicans* is a common member of the human gut flora. Fig. 7 presents the count of the living cells of *C. albicans* cells suspended in water and in the complex LB medium after different time exposure to activated argon plasma. The generated oxygen-argon plasma can successfully affect the *C. albicans* fungal cells with a reduction of approximately 95% in viable cell counts after a short exposure time of 10 s. However, the presence of LB medium components led to limited delay in lethal activity of Ar/O<sub>2</sub>. Hence, the D-value showed approximately 6.3 s increase in lethal activity due to the impact of organic materials as a component of LB nutritive medium.

It is interesting to note that there is a potential disinfecting capability of plasma derived from Ar or Ar/O<sub>2</sub> against *C. albicans*. Similarly, the effect of He/O<sub>2</sub> plasma on *C. albicans* biofilm was described by Sun

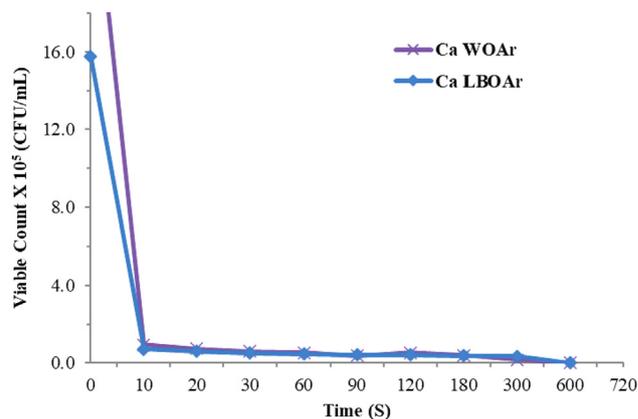


Fig. 7. *Candida albicans* cells exposed to argon plasma for varying periods of time while plasma is running at 14 kV applied voltage, 30 kHz frequency, and 4.5 slpm flow rate. The count were done for living cells in both water and LB medium.

et al. [65]. They explored an antifungal activity of cold plasma, and blended with antifungal drugs, against *C. albicans* biofilms. It was discovered that the plasma generated has antifungal effect and the generated ROS is the potential mechanism for that activity. Interestingly, oxygen argon plasma was advantageous especially when bacterial or fungal cells were suspended in LB organic medium, reflecting the promising use of this powerful plasma in disinfection especially in organic matter-rich medium.

In the current study, enhancement of argon plasma antimicrobial activity by enrichment with oxygen led to potential inactivation and disintegration of microbial cells. Increasing the efficiency of argon plasma by oxygen was recorded by Pignata et al. [66]. They recorded that oxygen argon Ar/O<sub>2</sub> plasma has more effect than plasma generated by pure argon Ar or pure oxygen alone, on *E. coli*, *Aspergillus brasiliensis* and pistachios. These results are supported by Colagar et al. [67]. They found that helium/oxygen plasma led to breakdown of DNA at different exposure periods. They assumed that exposure for longer than 20 s resulted in the complete degeneration of the DNA by fragmentation and smear formation on agarose gel.

As a major limitation in the current study, the influence of oxygen-argon cold plasma on bacterial and fungal cells on molecular level e.g. cell wall, membrane integrity, proteins, enzymes as and DNA genetic materials requires close investigation. Also, further studies are recommended to explore the effect of oxygen-argon cold plasma on biofilm-producing bacteria e.g. *Klebsiella pneumoniae*. Moreover, emphasis should be given to the effect of activated argon plasma on microbial pathogens existing in their natural habitats or in vivo.

#### 4. Conclusion

It is thought that the oxygen-argon atmospheric pressure-plasma is a prominent technology that can potentially decontaminate surfaces inhabited by *S. aureus*, *C. albicans*, and *E. coli* species. Even with organic material present in an exposed environment, the activated argon plasma remains functional. Due to enhanced antimicrobial activity of oxygen-argon plasma, it can be successfully used as an alternative drug to treat many diseases caused by antibiotic-resistant bacteria. On the other hand, factors such as duration of exposure as well as the presence of organic debris in exposure medium had significant impact on the lethal effect of oxygen-activated plasma. The current challenge is to improve the penetration power of oxygen-argon plasma in exposure medium.

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