JOURNAL OF APPLIED SCIENCES RESEARCH

ISSN: 1819-544X Published BY **AENSI Publication**
EISSN: 1816-157Xhttp://www.aensiweb.com/JASR
2017August;13(7):pages 28-34

Open Access Journal

Inactivation efficacy Staphylococcus aureus bacteria by a dielectric barrier discharge plasma jet

[1]FADHIL FULIFUL, [2]MOHAMMED KHALAF, [3]ADNAN ABBAS, [4]ALI MOHAMMED

[1,4]Department of Physics, University of Karbala, Karbala,Iraq.
[2,3]Center of Applied Physics Department, Ministry of Science and Technology, Baghdad, Iraq.
*[1]University of Karbala,Department of Physics, College of science, Karbala, Fadhil KhaddamFulifu*l.

Received 28 July 2017; Accepted 22 August 2017; Available online 12 October 2017

Address For Correspondence:
FADHIL FULIFUL,University of Karbala,Department of Physics, College of science, Karbala, Fadhil KhaddamFulifu*l*.
E-mail: Fadhil.khaddam@yahoo.com

ABSTRACT

A dielectric barrier discharge (DBD) plasma jet is widely used in different applications such as biomedical forsterilization, in this work**,** DBD plasma jet operated at atmospheric pressure and room temperature is designed. The inactivation efficiency of DBD plasma jet is evaluated against staphylococcus aureus bacteria with different time exposure. The effect of gas flow rate and irradiation time on the gas temperature is studied because ionized gas temperature is important factor in bacteria treatment. Optical emission spectroscopy (OES) is investigated to determine the plasma parameters such as electron temperature and electron density. Also, OES are used to detect the active species present inside the plume of plasma. Results showed that the applied voltage play an important role in increasing the electron density which increasing the amount of active species. The area of growth inhibition zone directly extendedwith increased the exposure time of DBD plasma jet. From these results, we believe that the main reasons for the inactivation of microorganisms are the reactive species (RONS) which creating from the surrounding air.

KEYWORDS:dielectric barrier discharge,plasma jet, plasma diagnostic, inactivation efficiency,Optical emission spectroscopy

INTRODUCTION

Non-thermal plasma is used in the health care for" processing" of medical tools and living tissues. The feature of tissue treatment with non-thermal plasma is non-destructive surgery, controlled, high-exactness removal of diseased sections. The advantages of the DBD jets are that the gas temperature of the plasma remains near to room temperature because the power density is low; also there are no risks of arcing on the sample which treat by plasma. These two advantages are very important for many applications especially in biomedical applications. In recent years, non-thermal plasmas have been extensively studied for several applications in bio-medical [1-3]. Atmospheric pressure plasma jets (APPJs) have indisputable advantages, such as environment friendly substances, smaller generator, more economical and high stability[4]. When APPJ is launched into air, it produces a large quantity of reactive oxygen and nitrogen species (RONS) under room temperature conditions[5]. Because of their rich reactive species, APPJs have become very attractive for many applications in biomedical such as bacterial inactivation, blood coagulation, cleansing of medical equipment, wound healing and applications in dentistry [6-10].Non-thermal plasma device type, a cylindrical DBD, which operated at atmospheric pressure used to develop a new decontamination technique for powdery foods, theinfluence of non-

ToCite ThisArticle:FADHIL FULIFUL, MOHAMMED KHALAF, ADNAN ABBAS, ALI MOHAMMED., Inactivation efficacy Staphylococcus aureus bacteria by a dielectric barrier discharge plasma jet.2017. **Journal of Applied Sciences Research**. 13(7);Pages: 28-34

thermal plasma treatment on taste components of the powder by using a SPME-GC/MS is inspected. The experimental results presented a positive sterilization of green tea powder [11].A DBD non-thermal helium plasma jet with a single electrode employed for inactivation of bacterial. Three types of bacteria (Gram-positive bacterium Enterococcus faecalis, Gram- negative bacterium Pseudomonas aeruginosa and the fungus Candida albicans) are used as targets. The presence of reactive species is established by optical emission spectroscopy and Scanning electron microscopy is applied for analysis of cell morphology. The microbicidal efficiency is estimated by determining the area of inhibition zone. Results showed the Gram-negative bacterium and the fungus is less susceptible to the plasma antimicrobial effects than the Gram-positive bacterium. Furthermore the formation of inhibition zone is non-homogenous when the flow rate increased[12].

In this study, a dielectric barrier discharge plasma jet device is designed. Optical emission spectrometer (OES) analysis is used to determine the plasma parameters such as the electron temperature and electron density. Also active particles presented inside the plume of plasma are detected by OES. The staphylococcus aureus bacterium is used as target to study the deactivation influence of DBD plasma jet on bacteria. The mechanism of bacterial inactivation in the DBD plasma jet is evaluated.

1.2. Figures:

1.2.1 DBD Plasma Jet Setup:

A DBD plasma jet device is designed, which generated at atmospheric pressure and room temperature. DBD plasma jet consisted of Pyrex tube as dielectric barrier discharge.

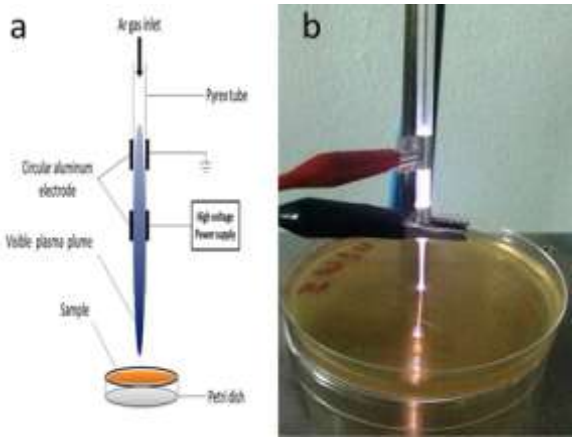

Fig. 1: (a) Schematic diagram and (b) photograph of the DBD plasma jet.

The length of the tube is 95mm with wall thickness of 0.925 mm, while the inner and outer diameters of the tube are 2 mm and 3.85 mm, respectively. Argon gas is fed at the upper of Pyrextube and controlled the flow rate by flow meters (Model/Part number: 11420 and Weight: 0.15 kg). Gas flow rate is fixed at 4 standard liter per minute (slm) of argon (commercial grade 99.999%).

DBD plasma jet device is based on a double-ring structureas shown in (Figure 1), having two aluminum rings shaped electrodes with a thickness of 0.1 mm and a width of 12 mm covered outer of the Pyrex tube. The distance between two electrodes is separated by 13 mm, while the downstream electrode is grounded and the upstream electrode is applied to power supply.

1.2.2 Instrumentation for Plasma Characterization:

BDB plasma jet is driven by using a home-made high voltage power supply with a frequency of 12 kHz and peak to peak voltage of 7.5 kV. The waveforms of the applied voltage and discharge current, as shown in (Figure 2), are recorded by a two-channel using PC USB Oscilloscope (Hantek6022BE, with a 20 MHz bandwidth and a 48 MS/s sampling rate), a high-voltage probe (Tektronix P6015) and a current probe (AT-C202).

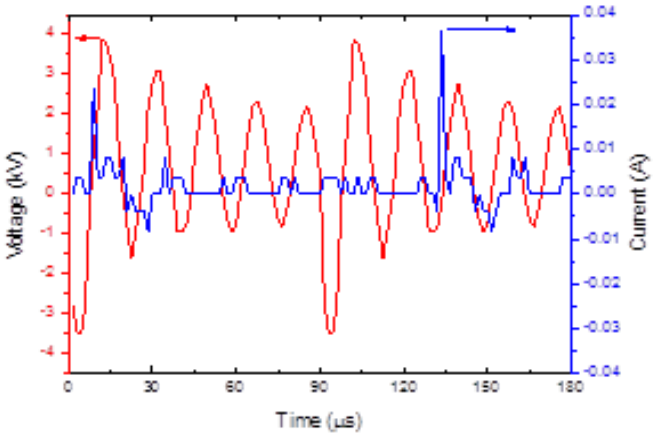

Fig. 2: Waveforms of the applied voltage and current

Figure 3 shows the plasma temperature against different flow rates, it is measured by thermometer using different flow rates at the distance of 2 cm from the nozzle to tube. The emission spectra of the DBD plasma jet is measured by spectrometer (SV2100, K-MAC) with the spectra range of 300 – 925 nm, whereas the optical fiber is located at 10 mm from the edge of Pyrex tube.

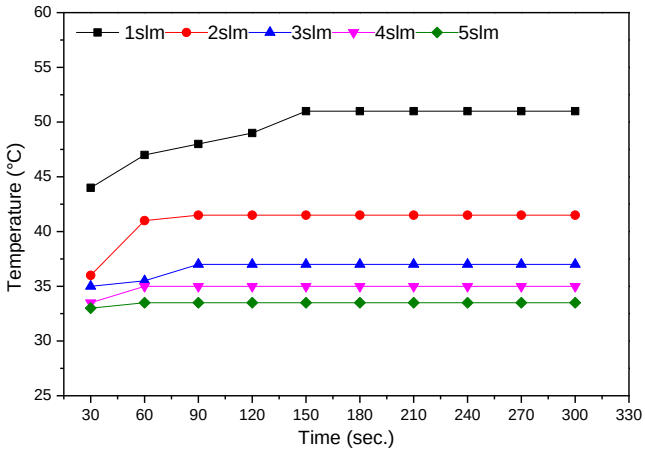

Fig.3: Plasma temperature with different flow rates

1.2.3 Microorganisms:

Bacterial suspension are Prepared for Staphylococcus aureus bacteria by a specific concentration. This concentration is measured by spectrophotometer at 625 nm and was 0.402 nm which is equivalent to a bacterial number of 6.5×10^{8} cells, compared to the McFarland solution. At 0.1 ml of cells are spread over the nutrient agar (medium) via spreading method by sterile swab and distributed in standard Petri dishes (90mm diameter). Petri dishes are kept in aseptic environment to dry for 10 min.

1.2.4 Plasma Treatment:

For all treatment, the DBD plasma jet generated at low frequency of 12 kHz with peak to peak voltage of 7.5 kV and argon flow rate is fixed at 4slm. The distance between the nozzle of Pyrex tube and the microorganisms is fixed at 20 mm. The bacteria are exposed to DBD plasma jet for different time intervals (1, 3, 5, 10 and 15 min). After treatment, all samples are incubated for 24 h at 37 °C. To determine the effect of plasma treatment on bacterial, the area of bacterial inhibition zones which formed on agar are measured by using Vernier callipers.

1.2.5 Optical Emission Spectroscopy (OES):

The information of active particles which present inside the plume of plasma can be obtained by analyzing its (OES). The spectral lines of the Ar DBD plasma jet under the peak to peak voltage of 7.5 kV is shown in (Figure 4).

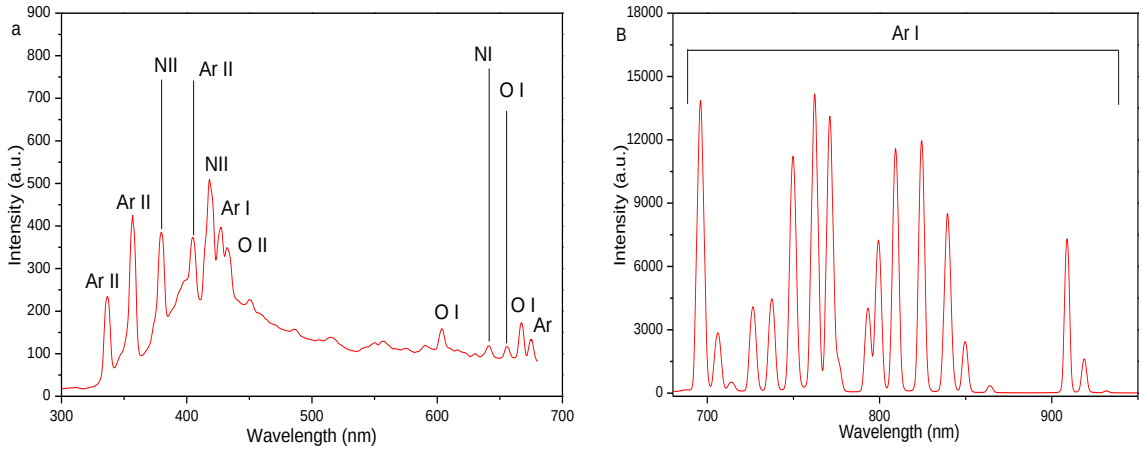

Fig. 4: Spectra of Ar DBD plasma jet (a) for wavelengths below 700nm and (b) for wavelength between 680 nm and 925 nm.

1.2.5.1 Diagnosis of electron temperature and electron density:

The electron temperature (T_e) and electron density (n_e) are important parameters to describe the characteristics of the Ar DBD plasma jet. For partial local thermodynamic equilibrium (PLTE) plasma[13], electron temperature can be determined by using a Boltzmann plot method[14, 15], given by:

$$\ln\left(\frac{I_{ij}\,\lambda_{ij}}{A_{ij}\,g_i}\right) = -\frac{E_i}{k_B T_e} \qquad (1)$$

Where I_{ij} is the relative intensity of the emitted line, λ_{ij} is wavelength, g_i is statistical weight, A_{ij} is transition probability, k_B is the Boltzmann constant and E_i is energy of the upper level.A graph is plotted for different values of $\ln\left(\frac{I_{ij}\,\lambda_{ij}}{A_{ij}\,g_i}\right)$ verses the energies of the upper level (E_i), give a straight line with a slope of $-\frac{1}{T_e}$.

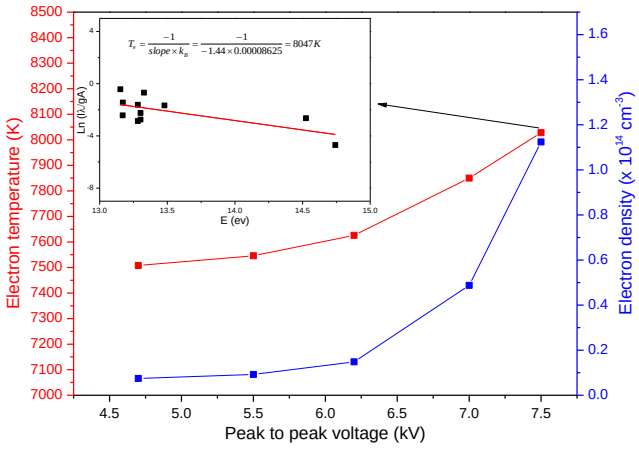

Fig. 5: Electron density and electron temperature as a function of peak to peak applied voltage. The inset graph is the Boltzmann's plot.

The electron density can be evaluated by using Saha-Boltzmann equation, given by[16]:

$$n_e = \frac{6.04\times10^{21} I_Z^*}{I_{Z+1}^*} T^{\frac{3}{2}} exp\left[\frac{-E_{k,z+1}+E_{k,z}-x_z}{k_B T_e}\right] cm^{-3} \qquad (2)$$

Where $I_Z^* = \frac{I_Z \lambda_{ki,z}}{g_{k,z} A_{ki,z}}$

In equation 2, the lower indices z denote the neutral particle and z+1 represent the singly charged ion and χ_z is the ionization energy of the species in the ionization stage Z. We measured two intensity ratios, 763.5 nm Ar(I) and 356.1 nm Ar(II) and electron density was estimated to be $n_e = 1.124 \times 10^{14}$ cm^{-3}.

1.3. Tables:

Table 1:SPECTROSCOPIC DATA OF AR-I EMISSION LINES USED IN BOLTZMANN PLOT[17].

λ_{ij} (nm)	A_{ij} (S^{-1})	g_i	E_i (ev)
763.5	2.45E+07	5	13.17
675.28	1.93E+06	5	14.742
696.54	6.39E+06	3	13.327
706.72	3.80E+06	5	13.302
714.7	6.25E+05	3	13.282
750.38	4.45E+07	1	13.479
772.37	5.18E+06	3	13.153
794.81	1.86E+07	3	13.282
800.61	4.90E+06	5	13.171
427.21	7.97E+05	3	14.5249

RESULTS AND DISCUSSION

(Figure 3) shows the gas temperature of DBD plasma jet at the distance from the nozzle of the tube of 2 cm with different flow rates at the plasma conditions of 12 kHz and peak to peak voltage of 7.5 kV. The results show that the gas temperature is 51 °C at argon gas flow rate of 1 slm, whereas the gas temperatures reached 33.5 °C at argon flow rate of 5 slm. For all flow rates the gas temperature reaches thermal equilibrium after 2-2.5 min of plasma exposure and the gas temperature remains the same up to 5 min. The emission spectra in the range of 300 nm to 925 nm are investigated. From (Figure4), the argon atoms were present in excited states and as ions. Also it can be seen that Nitrogen and Oxygen dominated at wavelength 300 nm until 700 nm which formed from the surrounding air and creationthe reactive oxygen and nitrogen species (RONS). The relationship between the electron density and electron temperature as a function of peak to peak applied voltage is shown in (Figure 6), where the electron density and electron temperature increased significantly with increased applied voltage.

(Figure 6) shows the bacterial zone of inhibition with different time exposure of DBD plasma jet. It was observed in (figure 6), that the sizeof inhibition zones was permanently larger than the diameter of the DBDplasma jet, this was due to the action of reactive oxygen species (ROS) which formed from surrounding air.

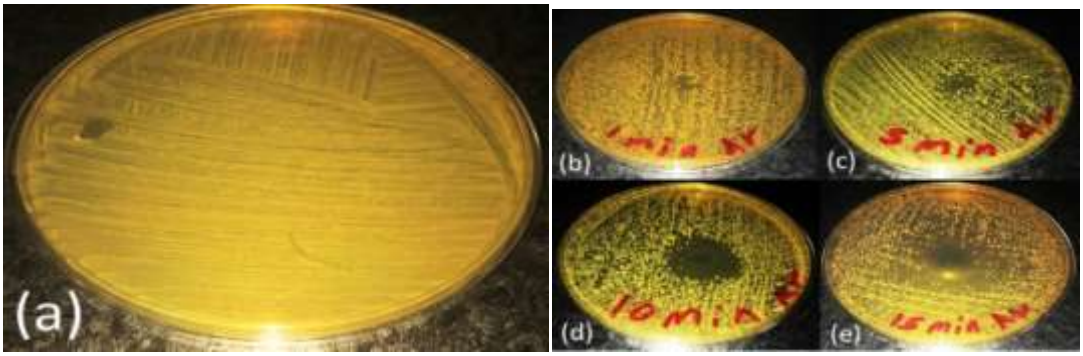

Fig. 6: Photographs of staphylococcus aureus samples on agar in petri dishes: (a) control sample, (b) 1 min, (c) 5 min, (d) 10 min, and (c) 15 min treated samples.

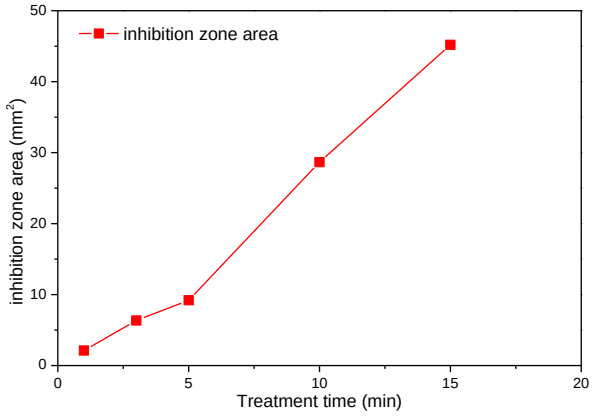

Fig. 7: Inhibition zone area as a function of treatment time.

The relationship between the areas of inhibition zone with different treatment time can be shown in (Figure7), where it is seen that the area of growth inhibition zone increased with increased the treatment time. From these results, we believe that the main reasons for the inactivation of bacteria were due the reactive species (RONS) which formed from the surrounding air.

Conclusions:

The Ar DBD plasma jet operated at atmospheric pressure and room temperature has beenconstructed and the inactivation of staphylococcus aureus microorganisms was investigated. The applied voltage play important role in determine the amount of active species and electron density. The diameter of growth inhibition zones on dishes is permanently larger than the plasma jet diameter. The area of growth inhibition zoneextends with increasing the exposure time of plasma jet and the inactivation rate of staphylococcus aureus cells is strongly dependent on the reactive oxygen and nitrogen concentration. Other gases can be used to generate DBD plasma jet at atmospheric pressure and room temperaturesuch helium gas, also it used in biomedical treatment.

REFERENCES

1. Xiaohu, L., H.Feng, G.Ying, Z.Jing andS.Jianjun, 2013. Sterilization of Staphylococcus Aureus by an atmospheric non-thermal plasma jet. Plasma Science and Technology, 15(5): 439.
2. Xu, Z., J.Shen, Z.Zhang, J.Ma, R.Ma, Y.Zhao andC.Cheng, 2015. Inactivation Effects of Non-Thermal Atmospheric-Pressure Helium Plasma Jet on Staphylococcus aureus Biofilms. Plasma Processes and Polymers, 12(8): 827-835.
3. Alkawareek, M.Y., Q.T.Algwari, G.Laverty, S.P.Gorman, W.G.Graham, D.O'Connell andB.F.Gilmore, 2012. Eradication of Pseudomonas aeruginosa biofilms by atmospheric pressure non-thermal plasma. PloS one, 7(8): e44289.
4. Du, C., Y.Liu, Y.Huang, Z.Li, R.Men, Y.Men and J.Tang, 2016. Qualitation and quantitation on microplasma jet for bacteria inactivation. Scientific reports, 6: 18838.
5. Nishime, T., A.Borges, C.Koga-Ito, M.Machida, L.Hein andK.Kostov, 2017. Non-thermal atmospheric pressure plasma jet applied to inactivation of different microorganisms. Surface and Coatings Technology, 312: 19-24.
6. Alkawareek, M.Y., S.P.Gorman, W.G.Graham andB.F.Gilmore, 2014. Potential cellular targets and antibacterial efficacy of atmospheric pressure non-thermal plasma. International journal of antimicrobial agents, 43(2): 154-160.
7. Kuwahata, H., T.Yamaguchi, R.-i.Ohyama andA.Ito, 2014. Inactivation of Escherichia coli using atmospheric-pressure plasma jet. Japanese Journal of Applied Physics, 54(1S): 01AG08.
8. Lee, M., H.Kim, Y.Kim, W.Y.Lee, K.Y.Baik, N.K.Kaushik andG.Cho, 2012. *Blood coagulation with atmospheric-plasma jets.* Paper presented at the Plasma Science (ICOPS), 2012 Abstracts IEEE International Conference on.
9. Xu, G.M., X.M.Shi, J.F.Cai, S.L.Chen, P.Li, C.W.Yao andG.J.Zhang, 2015. Dual effects of atmospheric pressure plasma jet on skin wound healing of mice. Wound Repair and Regeneration, 23(6): 878-884.
10. Weltmann, K.D., E.Kindel, T.von Woedtke, M.Hähnel, M.Stieber andR.Brandenburg, 2010. Atmospheric-pressure plasma sources: Prospective tools for plasma medicine. Pure and Applied Chemistry, 82(6): 1223-1237.

11. Hamajima, S., N. Kawamura and M. Nagatsu, 2017."Low-temperature disinfection of tea powders using non-equilibrium atmospheric pressureplasma,"inRecentGlobalResearchandEducation:Tech- nological Challenges, pp: 269-275.
12. Nishime, T., A. Borges, C. Koga-Ito, M. Machida, L. Hein and K.Kostov,2017. "Non-thermalatmosphericpressureplasmajetapplied to inactivation of different microorganisms," Surface andCoatings Technology,312: 19-24.
13. Ren, F., S.Ji, Z.Hao andY.Shi, 2016. Study of the Characteristics of Cold Ar Atmospheric Pressure Plasma Jet Generated With Inverted Tapered Tube. IEEE transactions on plasma science, 44(11): 2594-2602.
14. Förster, S., C.Mohr andW.Viöl, 2005. Investigations of an atmospheric pressure plasma jet by optical emission spectroscopy. Surface and Coatings Technology, 200(1): 827-830.
15. Gruenwald, J., J.Reynvaan, T.Eisenberg andP.Geistlinger, 2015. Characterisation of a Simple Non-Thermal Atmospheric Pressure Plasma Source for Biomedical Research Applications. Contributions to Plasma Physics, 55(4): 337-346.
16. Ley, H.-H., 2014. Analytical methods in plasma diagnostic by optical emission spectroscopy: A tutorial review. Journal of Science and Technology, 6(1).
17. Kramida, A., Y.Ralchenko and J.Reader, 2012. NIST Atomic Spectra Database (ver. 5.0)(online)(National Institute of Standards and Technology, Gaithersburg, MD.