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# OPTICAL EMISSION SPECTROSCOPY ANALYSIS OF ATMOSPHERIC PLASMA JET PLUME ON BACTERIA INACTIVATION

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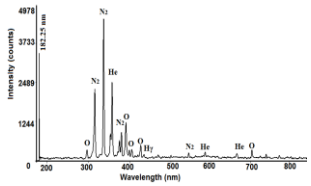
## Graphical abstract




Line emission spectrum recorded from spectrometer

## Abstract

In this paper, an atmospheric plasma jet plasma plume generated using Helium gas was investigated for reactive plasma species. The method of investigation is by using Optical Emission Spectroscopy analysis. Observation of the emission spectrum enables understanding of the influence of reactive species inside plasma plume to microbial inactivation process. The reactive species in plasma plume were detected using spectrometer without presence of bacteria. *Escherichia coli* and *Methicillin-resistant staphylococcus aureus* were used as inactivation targets. Bacteria were cultured in 10 Colony Forming Unit per milliliter in single colony and exposed to plasma at different time. It is found that, both bacteria were inactivated at 180 seconds. The result of emission line spectrum showed the presence of nitrogen and oxygen between line 300 nm until 700 nm. Nitrogen and oxygen are involved in oxidation process which is known as Reactive Nitrogen Species and Reactive Oxygen Species. These species are main key in bacteria inactivation.

*Keywords*: Atmospheric plasma jet; inactivation process; optical emission spectroscopy

## Abstrak

Dalam artikel ini, jet plasma yang dihasilkan dalam tekanan atmospheric menggunakan gas Helium telah disiasat berdasarkan spesies reaktif plasma. Spesies yang terhasil dalam kepulan dianalisis menggunakan 'optical emission spectroscopy'. Dengan memerhatikan pancaran spektrum, ia membolehkan pemahaman pengaruh spesies reaktif dalam proses menyahaktifan bakteria. Spesies reaktif dalam kepulan plasma dikesan dengan menggunakan spektrometer tanpa sampel bakteria. Dua jenis bakteria yang digunakan iaitu*Escherichia coli (E. coli)* dan *Methicillin-resistant staphylococcus aureus (MRSA)*. Bakteria yang dibiakkan dengan 10 Colony Forming Unit per mililiter dalam satu koloni dan didedah kepada plasma dengan masa yang berbeza. Di dapati bahawa, kedua-dua bakteria tidak aktif sepenuhnya pada 180 saat. Keputusan eksperimen garis pancaran spektrum menunjukkan nitrogen dan oksigen berada di antara 300 nm hingga 700 nm. Nitrogen dan oksigen terlibat dalam proses pengoksidaan yang dikenali sebagai 'Reactive Nitrogen Species' dan 'Reactive Oxygen Species'. Spesies ini mempunyai peranan utama dalam

penyahaktifan bakteria.

*Kata kunci*: Atmospheric plasma jet; inactivation process; optical emission spectroscopy



## 1.0 INTRODUCTION

Sterilization methods such as steam (autoclave), dry heat, flaming, chemical and radiation are widely used in the fields of medical, pharmaceutical, food and also environmental. Although these methods are effective, it is time consuming, dangerous to human (radiation exposes), high source requirement (electricity) and limited to specific material. As an alternative for these methods, atmospheric plasma is getting attention by researchers due its' ability in inactivation and sterilization purpose.

Plasma can be categorized into two types which is thermal and non-thermal plasma. Thermal plasma is more known as hot plasma or state of local thermodynamic equilibrium. Example of thermal plasma is natural plasma; lightning, aurora, sun, or human made; atmospheric arcs, spark or flame which uses high temperature especially to cut material [1].

This work focuses on non-thermal plasma or cold plasma, which are generated under atmospheric environment. Specific gas sources (argon, oxygen, helium) are supplied with high voltage (energy) to generate plasma. The plasma are generated when ionization and kinetic process occur in electrodes. The plasma size and shape depends on the setup device, power supply, gas source, flow gas and electrode conditioning. These differences will produce different number of species in plasma plume.

To create and utilize perfect plasma process, it is important to know all processes that take place in plasma plume and its properties. A method that can be used to investigate one of plasma property is optical emission spectroscopy (OES) analysis. In OES analysis, the spectrum emitted from plasma is measured as function of the wavelength. These spectra allow identification of the type of the excited particle in the plasma, the rotation and also the vibration temperature. OES allows to identify many radicals and active atomic or molecular species and also give insight in the plasma chemical process [2].

In this work, OES analysis is used to determine the elements present inside plume. These elements consist of active species, charge particle, UV photon, radical, excited electrons, and also electrical field [3]. Every single elements in atmospheric plasma is able to generate numerous characteristics for inactivation purpose [4]. Therefore, these characteristics make plasma very reliable as disinfection device especially in biomedical industry. Although the plasma jet is relatively in small size, it was appropriate for our purpose research in understanding the microbial inactivation. This small size of device also allows very precise treatment which can reduce the damage to the surrounding of healthy living cells or tissue [5].

## 2.0 EXPERIMENTAL

### 2.1 Atmospheric Plasma Jet Device

The experimental arrangement is shown in Figure 1. The set up composed of fours mains parts; gas control system, pulse inverter, plasma jet setup, and spectrometer device. The gas control system includes the flow meter system, valve, power system, control system, gases and Labview® (2011) software. Plasma jet setup consists of two electrodes where the ionization occurs. These electrodes are placed 2 cm between each other on the glass tube (diameter 1.50 mm). Helium gas with flow rate 1000ml/min are used as a main gas source.

The emission line spectrums for plasma jet plume were measured using EPP2000-HR High Resolution spectrometer from Stellar. The plume was collected by using fiber optic probe (UV-VIS-NIR) with core diameter 600 µm. The fiber optic probe was held 0.5 mm away from plume at 45°. The recorded spectrum line was observed using SpectraWiz OS v5.3(c) 2013 software.

### 2.2 Bacteria Preparation and Exposure to Plasma

Bacteria samples were obtained from Microbiology Laboratory, Tuanku Fauziah Hospital, Malaysia. *E. coli ATTC* 25922 and wild *MRSA* were prepared in concentration of 10 colony forming units per milliliter (CFU/ml) in Mueller Hinton agar medium in single colony as shown in Figure 2 (a) and (b). Every colony was cultured approximately the same size in order to have homogeneous sample size for plasma treatment.

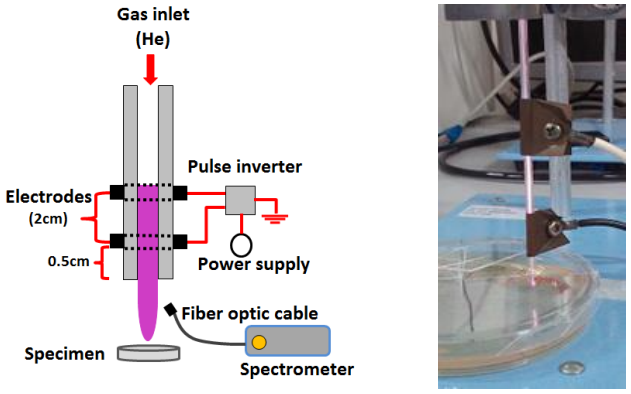
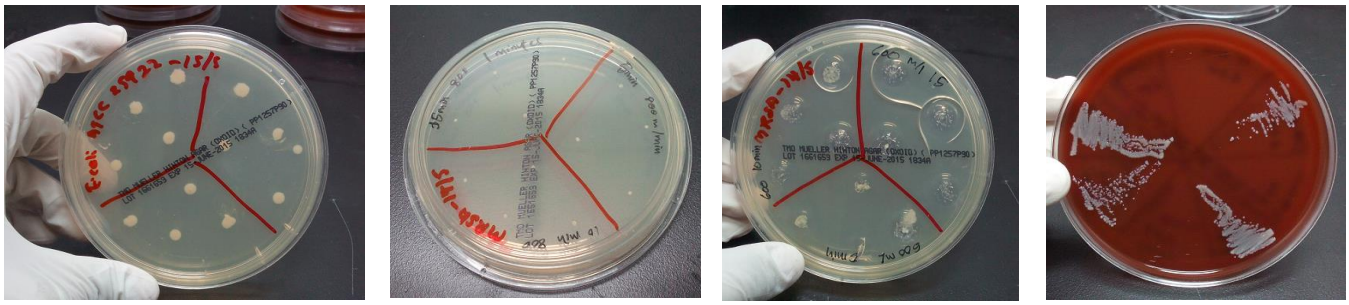



**Figure 1** Plasma jet device and plasma when exposed to sample.



(a) (b) (c) (d)

**Figure 2** (a) Bacteria *E. coli* ATTC 25922 (b) bacteria *MRSA*, (C) *E. coli* after plasma treatment and (d) *MRSA* growth in Blood agar to monitor the effectiveness of plasma treatment.

The sample bacteria were placed under plasma plume and treated at different time treatment. After exposed to plasma, sample were re-culture and incubated for 24 hours at 37°C in MacConkey (for *E. coli*) and Blood agar medium (for *MRSA*) as shown in Figure 2 (c) and (d). This was to monitor the effectiveness of treatment.

## 3.0 RESULTS AND DISCUSSION

### 3.1 Optical Emission Spectroscopy

The emission spectra in the range of 200 nm to 800 nm were recorded and analyzed. From Figure 3, it was determined that Nitrogen and Oxygen dominated at wavelength 300 nm until 700 nm. Atomic emission line of nitrogen are 308.52 nm (N III), 315.08 nm (N V), 336.13 nm (N III), 379.76 nm (N III), 545.57 nm (N II), 726.22 nm (N II), and 742.53 nm (N I) were identified. Oxygen is present at Oxygen 398.81 nm (O II), 405.03 nm (O II), 426.83 nm (O II), 705.31 nm (O IV), and 296.29 nm (O II). Hydrogen, Hγ is present at line 434.41 nm. Apart from Helium as carrier gas, the presence of other active species and atomic is expected of the back diffusion of the ambient air [6].

In plasma plume, the atoms constantly experience collisions with each other, leading to excitations to the various possible energy levels. The presence of Helium molecule in atmosphere causes the expanding process with surrounding air which leads to NO and $N_2$ (namely the γ-system and the second positive system) production [7]. This chemical and physical reaction produced the reactive oxygen such as O, OH, and $O^*_2$.

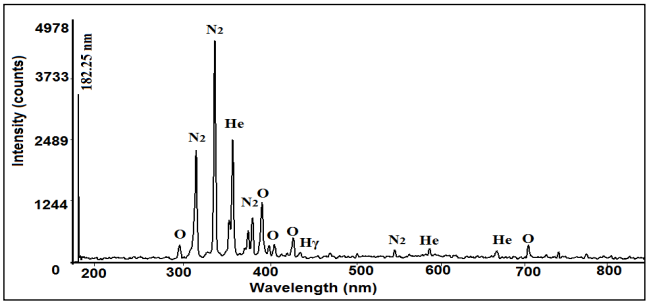



**Figure 3** Line emission spectrum recorded from spectrometer

$$He + electron \rightarrow He^*(2^3S, 2^1S) + electron \quad (1)$$

$$He^* + O \rightarrow He + O + e \quad (2)$$

$$He^* + O_2 \rightarrow He + O^* + O \quad (3)$$

The expanding process in plume can be explained by removal of electron from Helium atoms. The Helium lines with spectral properties are shown in Table 1. The upper level energy is from range 23 eV to 51 eV. This is enough energy required to remove the first electron in Helium atoms (~24.6eV) [8]. The removal of electron in atoms will lead to generation of other-species.

Nitrogen was detected with high intensity in line 300 nm to 400 nm. In open air experiment, nitrogen experience the electron induce process and generated nitrogen atoms as in (4) [9]. The presence of N in open air also can lead to production of NO species [6].

$$e + N2 \rightarrow N + N + e \quad (4)$$

$$N + O + N_2 \rightarrow NO + N_2 \quad (5)$$

$$e + N_2 \rightarrow N\bullet + N + e \quad (6)$$

$$NO + O_3 \rightarrow NO + 2O_2 \quad (7)$$

$$e + O_2 \rightarrow 2O + e \quad (8)$$

$$O + O_2 \rightarrow O_3 \quad (9)$$

Open air experiment can create some reaction pathways that lead to more generation of active species due to present of humidity [7][10][11]. The water ($H_2O$) molecule dissociate with collision of electrons, non-radical (ozone, $O_3$) or other species such as helium metastable (He*) that can produce the Hydroxyl (OH) radical, $O_2$ and H molecule.

**Table 1** Helium transitions used for the determination of electron excitation temperature and electron density [12].

| $\lambda_{ki}$ (nm)[a] | Transitions | $E_k$ (eV)[b] | $E_i$ (eV)[c] | $A_{ki}$ ($s^{-1}$)[d] |
|---|---|---|---|---|
| 356.81 | $10s^3S$ —$2p^3P$ | 24.443 | 20.964 | 2.6868E+05 |
| 468.86 | $4p^2P$ — $3d^2D$ | 51.016 | 48.371 | 5.5636E+06 |
| 501.24 | $3p^1P$ — $2s^1S$ | 23.087 | 20.615 | 1.3372E+07 |
| 586. 99 | $3d^1D$ —$2p^3P$ | 23.074 | 20.964 | 4.310E+03 |
| 666.88 | $3d^1D$ —$2p^1P$ | 23.074 | 21.218 | 6.3705E+07 |

[a] $\lambda_{ki}$ is the wavelength in nanometer of helium.
[b] $E_k$ (eV) upper level energy in electron volts.

[c] $E_i$ (eV) lower level energy in electron volts.
[d] $A_{ki}$ ($s^{-1}$) is transition probability.

$$H_2O + O_3 \leftrightarrow O_2 + 2OH \quad (10)$$
$$H_2O + O_3 \rightarrow O_2 + H_2O_2 \quad (11)$$
$$2H_2O \rightarrow H_2O_2 + H_2 \quad (12)$$
$$H_2O_2 \rightarrow 2OH\bullet \quad (13)$$
$$e + H_2O \rightarrow OH\bullet + H\bullet + e \quad (14)$$

The presence of hydrogen atomic, Hγ ($5d^2D$ — $2p^2P$) are due to excitation of $H_2O$ molecule during ionization process.

$$e + H_2O \rightarrow OH + H + e \quad (15)$$
$$H + O_2 \rightarrow OH + O \quad (16)$$
$$H + O + He \rightarrow OH + He \quad (17)$$
$$H + NO_2 \rightarrow NO + OH \quad (18)$$
$$OH + O \rightarrow H + O_2 \quad (19)$$

There also presence of ultra-violet (UV radiation observed at line 182.25 nm in category UV-C (200-280nm). This UV presence in region with doses of several miliwatt per square centimeter can causes minor damage on the bacteria [10][13].

### 3.2 Bacteria Inactivation

This work experimented on two types of bacteria which is *E. coli* (ATTC 25992) and *MRSA* (from patient). The bacteria were prepared in concentration of 10 CFU/ml. The plasma treatment result for *E. coli* and *MRSA* are shown in Table 2. *E. coli* was inactivated by plasma helium faster than *MRSA*. One of the differences between *E. coli* (gram negative) and *MRSA* (gram positive) can be explained by cell wall structure. The results from this experiment are in agreement with several researches that state that gram negative bacteria are inactivated faster compared with gram positive bacteria [14-16]. Best result of inactivation is achieved at exposure time 3 minutes and above.

**Table 2** Disinfection of bacteria *E. coli ATTC* 25922 and *MRSA*

| Bacteria | Time treatment (seconds) | | | | |
|---|---|---|---|---|---|
| | 60 | 120 | 180 | 240 | 300 |
| *E. coli* | / | X | X | X | X |
| MRSA | / | / | X | X | X |

[a] X : no growth of bacteria
[b] / : growth of bacteria

### 3.3 Active Species and Their Role in Inactivation Process

Active species in inactivation of microbe has been investigated by many researchers in order to understand the ability of plasma to sterilize or inactivate microbe. In the emission line of helium plasma, nitrogen and oxygen are identified as the key of inactivation process. It is recognized as reactive oxygen species (ROS) and reactive nitrogen species (RNS) give high impact inactivation microbe [9] . ROS and RNS consists of O, $O_3$, $O_2$, OH, N, $N_2$, and NO effect on biomolecules molecular (OH, NO) and atomic (H, O, N) radicals [11]. They act together to penetrated inside microbe and damage the components which causes by nitrosative stress [17].

ROS and RNS are involved in the oxidation and reduction process on the microbe. Plasma supply large oxidants compounds to sample causes damage to cells and tissues and make various antioxidant defense mechanisms become depleted [17]. RNS and ROS generally give impact on the nucleic acids, protein and lipids [18]. The chromosomal inside DNA become unstable, mutations, loss of organelle functions and causes membrane damage (lipids layer)[5][18]. The structure of microbial structure was damaged by lipid peroxidation.

The inactivation of both bacteria can be explained by the diffusion of reactive species through cell wall [10]. Bacteria *MRSA* have thicker peptidogly layer which make it high resistive in receive physical disturbance [19]. Elements in plasma plume are easier to penetrate *E. coli* membrane which only consists of single layer of peptidoglycan. Therefore, longer treatment can provide higher quantity of RNA and ROS inside wall and affected as damage to bacteria. Penetration of elements in plume are also been affected by other factor such as surface area of treatment, bacteria concentration, and strain.

Other components such as mitochondrion, ribosome, and cell membrane are also damaged due to effect of other elements in plume. If the mitochondria are unable to control sugar and lipid, metabolism in ATP production will limit the energy production and make cell dies due to apoptosis or necrosis [17]. The ozone present in emission will create intrusion with cellular respiration [10]. Electrical field on the plasma plume also can affect the membrane cell that caused the electrostatic disruption or at least permeabilization for a very short time [20]. Ions inside plasma plume can cause acidified on the hydrated surface of microbe. This process of hydrated involve the reaction by nitride oxide (NO, $NO_2$) and OH radical (nitrous acid $HNO_2$ and nitric acid $HNO_3$) [7].

The research will need further study for wider application especially in biomedical field. There are still some features that we do not understand such as kinetic and collision process, density and also temperature of electron inside plasma jet. Special setup analysis and calculation are required in order to investigate those features. Furthermore, how to improve the ability device for other application is a topic of ongoing and further research work.

## 4.0 CONCLUSION

In this paper, the active species inside atmospheric plasma plume were identified using optical emission spectroscopy analysis. It was found that, nitrogen dominated in emission lines 300 nm until 400 nm. The

reactive nitrogen species and reactive oxygen species present crucial role in this inactivation experiment. These species causes damage by providing abnormal load oxidation process to bacteria. This then, will cause death to *E. coli* and *MRSA* due to mutation and dysfunction of components inside the bacteria. Other elements inside plume such as ions, electrical field and UV plays secondary role but still important in inactivation purpose. The developed atmospheric plasma jet device is comparatively a simple device which possesses good potential as microbial inactivation device since it is able to inactivate the pathogen in short time.

## Acknowledgement

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## References

[1] Conrads, H., and M. Schmidt. 2000.Plasma Generation and Plasma Sources. *Plasma Sources Science and Technology*. 9(4): 441.

[2] Machala, Z., Janda, M., Hensel, K., Jedlovský, I., Leštinská, L., Foltin, V., Morvová, M. 2007. Emission Spectroscopy of Atmospheric Pressure Plasmas for Bio-Medical and Environmental Applications. *Journal of Molecular Spectroscopy*. 243(2): 194–201.

[3] Kong, M. G., Kroesen, G., Morfill, G., Nosenko, T., Shimizu, T., van Dijk, J., and Zimmermann, J. L. 2009.Plasma Medicine: An Introductory Review. *New Journal of Physics*. 11(11): 115012.

[4] Fridman, G., Friedman, G., Gutsol, A., Shekhter, A. B., Vasilets, V. N., and Fridman, A. 2008. Applied Plasma Medicine. *Plasma Processes and Polymers*. 5(6): 503–533.

[5] O'Connell, D., Cox, L. J., Hyland, W. B., McMahon, S. J., Reuter, S., Graham, W. G., Currell, F. J. 2011.Cold Atmospheric Pressure Plasma Jet Interactions with Plasmid DNA. *Applied Physics Letters*. 98(4): 043701.

[6] Anghel, S. D., and Simon, A. 2010. Preliminary Investigations of a Very Low Power Atmospheric Pressure Helium Plasma. *Rom. Journ. Phys* .55(1-2): 185–193.

[7] Weltmann, K.-D., Brandenburg, R., von Woedtke, T., Ehlbeck, J., Foest, R., Stieber, M., and Kindel, E. 2008. Antimicrobial Treatment of Heat Sensitive Products by Miniaturized Atmospheric Pressure Plasma Jets (APPJs). *Journal of Physics D: Applied Physics*. 41(19): 194008.

[8] "Basic Atomic Spectroscopic Data," *National Institute of Standards and Technology*, [Online]. From: http://physics.nist.gov/PhysRefData/Handbook/Tables/heliumtable1.htm. [Accessed on16 June 2015].

[9] Murakami, T., Niemi, K., Gans, T., O'Connell, D., and Graham, W. G. 2013 . Chemical Kinetics and Reactive Species in Atmospheric Pressure Helium–oxygen Plasmas with Humid-Air Impurities. *Plasma Sources Science and Technology*. 22(1): 015003.

[10] Laroussi, M., and Leipold, F. 2004. Evaluation of the Roles of Reactive Species, Heat, and UV Radiation in the Inactivation of Bacterial Cells by Air Plasmas at Atmospheric Pressure. *International Journal of Mass Spectrometry*. 233(1-3): 81–86.

[11] Marcelo Hermes-Lima, 2005. Oxygen in Biology and Biochemistry: Role of Free Radicals. In Functional Metabolism: Regulation and Adaptation Pp. 319–368. Wiley Online Library. http://www.geocities.ws/hermeslima_etal/chapter12.pdf.

[12] Yuri Ralchenko, Alexander Kramida, and Joseph Reader, "NIST atomic spectra database (version 5), [Online]. From: http://www.nist.gov/pml/data/asd.cfm. [Accessed on16 June 2015].

[13] Chen, W., Huang, J., Du, N., Liu, X.-D., Wang, X.-Q., Lv, G.-H., and Yang, S.-Z. 2012. Treatment of Enterococcus Faecalis Bacteria by a Helium Atmospheric Cold Plasma Brush with Oxygen Addition. *Journal of Applied Physics*. 112(1): 013304.

[14] Tipa, R. S., Boekema, B., Middelkoop, E., & Kroesen, G. M. W. (n.d.), "Cold Plasma for Bacterial Inactivation." Retrieved from http://www.ispc-conference.org/ispcproc/ispc20/312.pdf, [Accessed on 10 June 2015.

[15] Sharkey, M. A., Chebbi, A., McDonnell, K. A., Staunton, C., and Dowling, D. P. 2015. Evaluation of the Sensitivity of Bacterial and Yeast Cells to Cold Atmospheric Plasma Jet Treatments. *Biointerphases*. 10(2): 029507.

[16] Fröhling, A., Baier, M., Ehlbeck, J., Knorr, D., and Schlüter, O. 2012. Atmospheric Pressure Plasma Treatment of Listeria Innocua and Escherichia Coli at Polysaccharide Surfaces: Inactivation Kinetics and Flow Cytometric Characterization. *Innovative Food Science & Emerging Technologies* .13: 142–150.

[17] Klandorf, H., and Van Dyke, K. 2012. Oxidative and Nitrosative Stresses: Their Role in Health and Disease in Man and Birds. INTECH Open Access Publisher. http://cdn.intechopen.com/pdfs/35982.pdf, [Accessed on 4 June 2015.

[18] Waris, G., and Ahsan, H. 2006. Reactive Oxygen Species: Role in the Development of Cancer and Various Chronic Conditions. *Journal of Carcinogenesis*. 5(1): 14.

[19] Kenneth Todar, "Structure and Function of Bacterial Cells," *Todar's Online Textbook of Bacteriology*, [Online]. From: http://www.textbookofbacteriology.net/structure_5.html. [Accessed on 4 June 2015.

[20] Klampfl, T. G., Isbary, G., Shimizu, T., Li, Y.-F., Zimmermann, J. L., Stolz, W., Schmidt, H.-U. 2012. Cold Atmospheric Air Plasma Sterilization against Spores and Other Microorganisms of Clinical Interest. *Applied and Environmental Microbiology*. 78(15): 5077–5082.