Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Electrostatics 64 (2006) 17–22
Journal of ELECTROSTATICS
www.elsevier.com/locate/elstat

Analysis of sterilization effect by pulsed dielectric barrier discharge

Jai Hyuk Choi[a,*], Inho Han[a], Hong Koo Baik[a,*], Mi Hee Lee[b], Dong-Wook Han[b], Jong-Chul Park[b], In-Seob Lee[c], Kie Moon Song[d], Yong Sik Lim[d]

[a]*Department of Metallurgical Engineering, Yonsei University, 134 Shinchon, Seodaemun, Seoul 120-749, Republic of Korea*
[b]*Department of Medical Engineering, Yonsei University College of Medicine, 134 Shinchon, Seodaemun-ku, Seoul 120-752, Republic of Korea*
[c]*Atomic-Scale Surface Science Research Center, Yonsei University, 134 Shinchon, Seodaemun-ku, Seoul 120-749, Republic of Korea*
[d]*Department of New Material Science Applied Physics, Konkuk University, 380-701 Chungju, Republic of Korea*

Received 19 January 2005; received in revised form 17 March 2005; accepted 22 May 2005
Available online 28 June 2005

Abstract

Atmospheric pressure (AP) plasmas can sterilize against almost all kinds of bacteria because many ions and reactive species, such as oxygen atoms and ozone, etc., are generated during AP plasmas. So AP plasmas are proper processes for application to air cleaners and sterilizers. The aim of this paper is to evaluate a germicidal effect caused by pulsed plasma system in air utilizing a dielectric barrier discharge (DBD) type reactor incorporating alumina, glass, etc. *Escherichia coli*, *Bacillus subtilis* and *Pseudomonas aeruginosa* bacteria were used for this sterilization experiment. For analysis of the relationship between sterilization results and chemical species generated in the discharge, we used optical emission spectroscopy and we checked emission spectra by atomic oxygen (394.2 and 436.8 nm) and second positive system of nitrogen (337.1 nm). Experimental results showed that DBD treatment during 70 s sterilized *E. coli* with 99.99% effectively and ozone molecules were the dominant germicidal species. From these results we concluded that the pulsed DBD system is very effective for sterilization.

Keywords: Plasma; Sterilization; Dielectric barrier discharge

1. Introduction

Atmospheric pressure (AP) plasmas have been developed for many applications such as printed circuit board (PCB) hole etching [1,2], surface modification of polymers [3–7], air purification [8–11] and sterilization [12–23]. Nowadays, the research on plasma sterilization is on the rise.

Sterilization is a physical or chemical process that impairs or eliminates microorganisms, especially bacteria. For a long time, researchers have reported that plasma can kill or inhibit the growth of bacteria. Many sterilization methods such as autoclaving, γ-irradiation, ethylene oxide (EtO) and UV sterilization, as well as plasma sterilization are currently being used [13].

However, most means for sterilization, except AP plasma, need lengthy sterilization time (~30 min) and can be operated only in closed space while other methods are toxic to the human body; these are not easy to apply to air purifiers for germicidal effect. Thus, AP plasmas that can be applied to open space, have very short sterilization time (~1 min), and generate many reactive species (e.g., ozone, hydroxyl radical and oxygen atom.), have huge merit for application to sterilization systems.

Generally, microorganisms are sterilized by physical or chemical processes in AP plasma. The physical process proceeds by positive and negative ions in the discharge's streamer, and chemical process is done by ozone, atomic oxygen, hydroxyl radical, etc.

Montie [21] reported that plasma sterilization could be classified in three mechanisms; the hydroxyl radical can attach to unsaturated fatty acids and induce lipid peroxidation; oxygen radical can cause DNA oxidation;

*Corresponding authors. Tel.: +82 2 2123 2838; fax: +82 2 312 9846.
E-mail address: thinfilm@yonsei.ac.kr (H.K. Baik).

0304-3886/$ - see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.elstat.2005.04.001

and oxidation of amino acids can occur followed by protein oxidation. Laroussi [22] reported that fatty acid peroxide was formed by plasma and it altered to the membrane lipids. But sterilization did not proceed by only chemical effect. Mendis [23] stated that charge accumulation on the cell membrane induced electrostatic stress which ruptured cells. After all, the sterilization mechanism using plasma has been researched in many prior papers but is still unclear. So we focused on the exact sterilization mechanism in this paper.

Among the several plasma types, dielectric barrier discharge (DBD) is most frequently used. DBD arrangements are characterized by a dielectric layer covering at least one of the electrodes. Our main purpose is to report how we produce an efficient sterilization system utilizing pulsed DBD and analyzed its germicidal effect by optical emission spectroscopy (OES).

2. Experiment

Fig. 1 shows the experimental apparatus schematically. As a negative DC pulse is applied, DBD is generated in the zone between two planar electrodes, each of which is covered with an alumina dielectric layer. Fig. 2 shows the current I and voltage V waveform

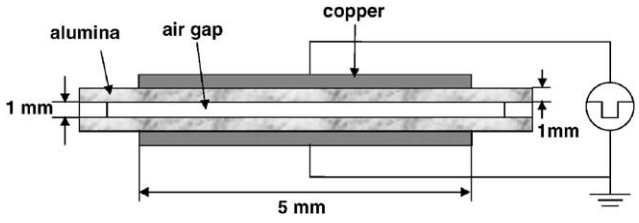

Fig. 1. Schematic diagram of atmospheric pressure DBD reactor (edge view of planar geometry).

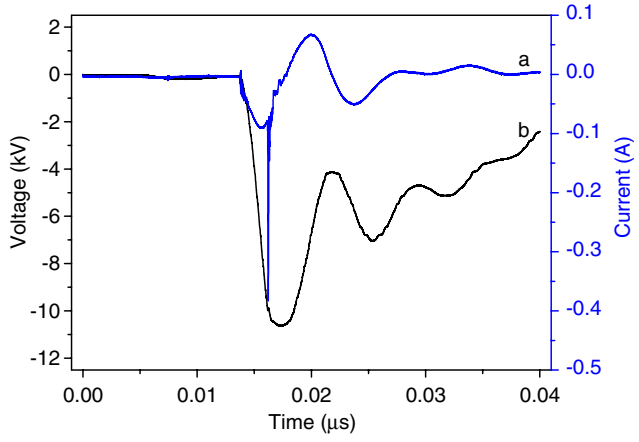

Fig. 2. Current and voltage waveform of negative DC pulse discharge across DBD reactor. (a) Current, (b) voltage.

of the negative DC pulse discharge. Generally DC pulse is more efficient than AC power because DC pulse can make higher reduced electric field E/N (where E is electric field and N is the gas density) than AC. Consequently, more energetic electrons can be generated having a higher electron energy distribution function (EEDF) [24]. The pulse was generated by thyratron switching element and the pulse rise time is about 3 μs and applied frequency is 1 kHz. Both electrodes had the discharge area with 50 mm × 20 mm with 1 mm gap spacing and the dielectric material is alumina of 1 mm thickness.

For the evaluation of germicidal effect by pulsed DBD plasma treatment, we used the cell counting method [13]. First *Escherichia coli* was suspended in 0.9% normal saline. Suspensions of the bacterial spores were inoculated onto circular cover glasses having the radius of 6 mm and the thickness of 0.13 mm, from Marienfeld Company. After drying at room temperature for 2 h, cover glasses were slid into the air gap of the reactor and rested on the lower alumina plate for DBD treatment. After plasma treatment, cover glasses were put in the normal saline and stirred for 10 min for removal and dispersion of *E. coli* colonies. After 1/10 dilution, colonies in the saline were spread over a standard agar plate. The number of bacteria colonies (expressed as colony-forming units CFU) was counted after 1 day of incubation at 37 °C for the bacteria.

For the observation using a scanning electron microscope (SEM) of the morphologies of the *E. coli* cell before and after plasma treatment, we coated poly L-lysine on cover glasses to develop adhesion between cells and glass. The *E. coli* samples were plasma-treated for five different treatment time of 0, 10, 30, 50 and 70 s and the replication numbers at each condition are three. The cover glasses, both treated and untreated controls, were coated with an ultra-thin layer of gold by ion sputter and the morphologies observed by SEM (s-800, HITACHI, Tokyo, Japan).

3. Result and discussion

Fig. 3 shows variations of *E. coli* colony densities versus the treatment time for 1 kHz negative pulse discharge of 11 kV. As seen the number of CFU decreased logarithmically, we concluded that plasma can effectively sterilize the *E. coli* bacteria with adequate discharge time. Generally, kinetics of sterilization processes are described by the number of surviving CFU vs. plasma treatment time and can be plotted by three categories; single slope, double slope and multiple slope by D-value [16–23]. D-value is defined as the time required to reduce an original concentration of cells by 90% and therefore short D-value indicates good sterilization efficiency.

Among all these cases, sterilization results for single slope were reported most frequently because the increase of discharge time means increase of germicidal doses.

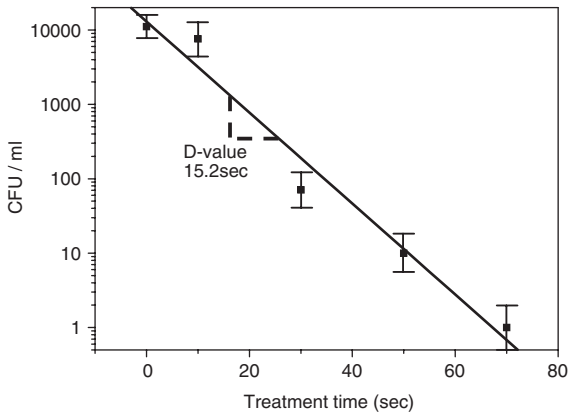

Fig. 3. Linear-fitted surviving number of CFU of *E. coli* versus the treatment time for negatively pulsed DBD of 11 kV at 1 kHz. The replication numbers are three and the bars are 'standard error bars'.

From Fig. 3, our sterilization results also showed the single slope and the *D*-value in our experiment was about 15.2 s.

Figs. 4 and 5 show the SEM images of *E. coli* at each experimental condition. The images in Fig. 4 were magnified 2000 times and images in Fig. 5 were 10,000 times. These images indicate that plasma treatment breaks up *E. coli* colonies and erodes the spore surface or splits into many pieces. These experiments also showed the same results that sterilization effect by plasma increases with treatment time.

From these references we can conclude that the sterilization effect increased with treatment time by not only physical damage caused by streamer but also by chemical damage by most reactive neutral species. In order to determine whether the physical or the chemical process affected microorganisms during AP plasma treatment, the following sterilization tests and the experimental procedure were conducted.

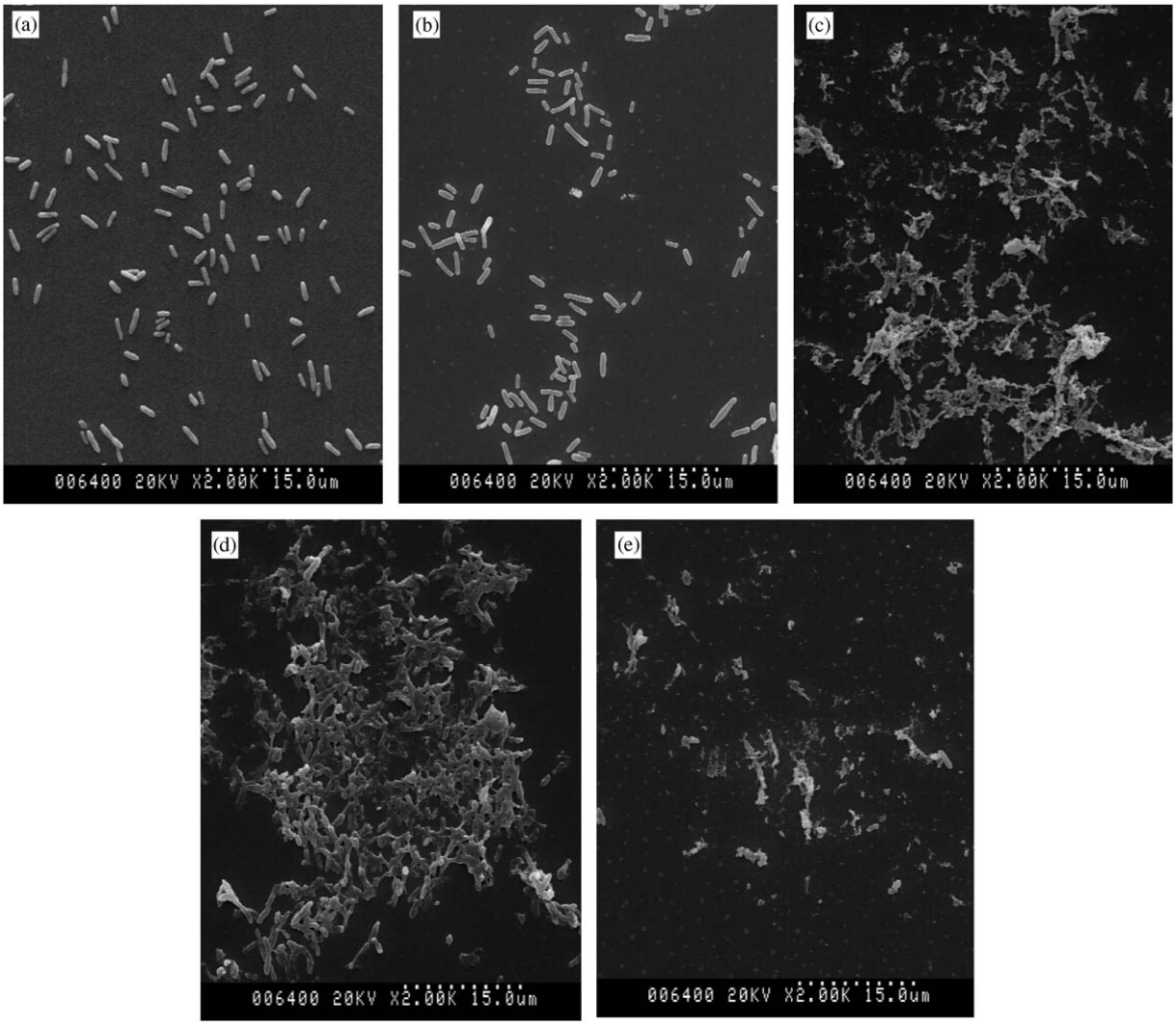

Fig. 4. SEM images of *E. coli* at each duration time for treatment in AP plasma (2000 × magnification). (a) 0 s (b) 10 s (c) 30 s (d) 50 s and (e) 70 s.

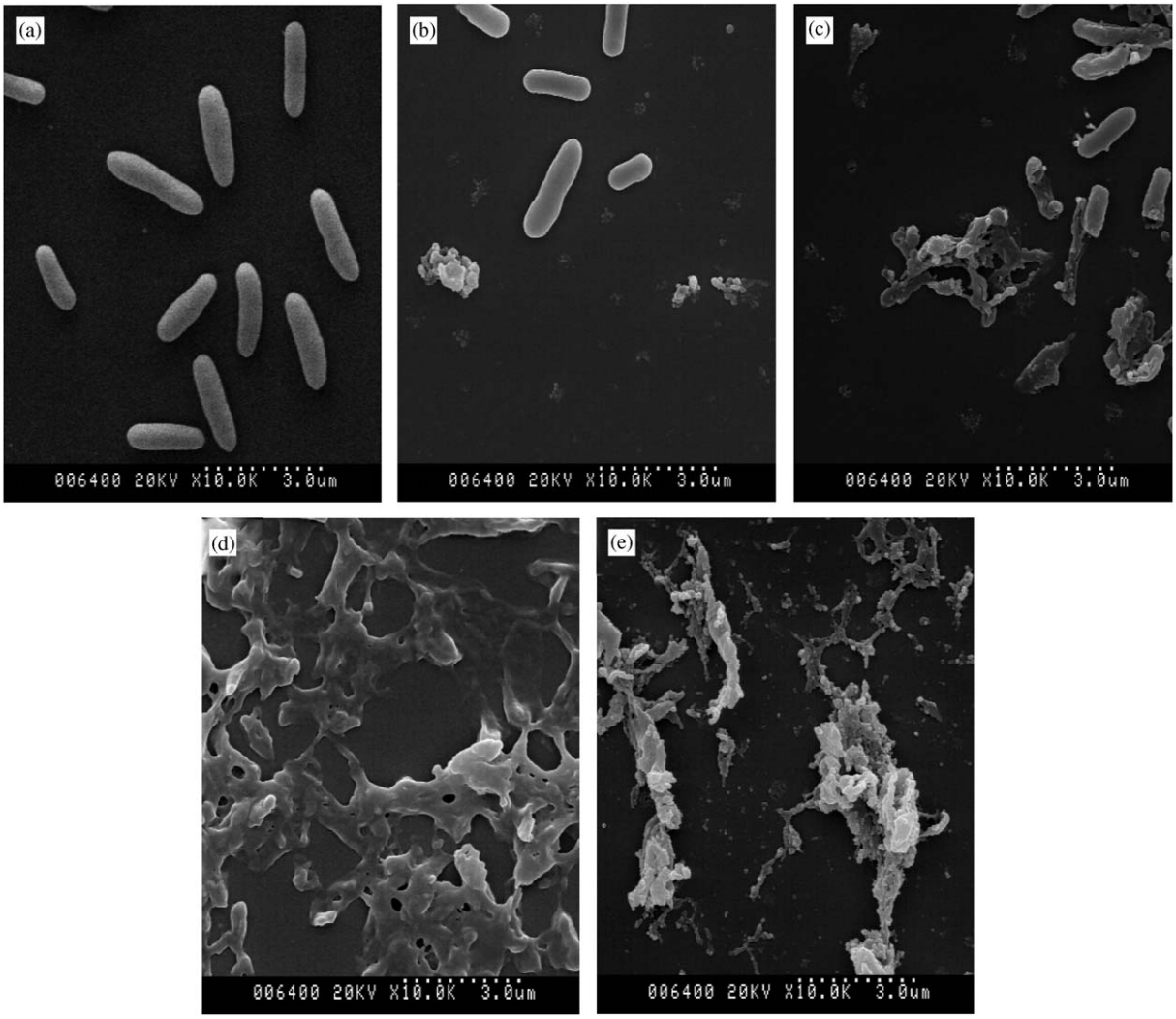

Fig. 5. SEM images of *E. coli* at each duration time for treatment in AP plasma (10,000 × magnification). (a) 0 s (b) 10 s (c) 30 s (d) 50 s and (e) 70 s.

Agar medium was plated in a Petri dish and then three bacteria, *E. coli*, *Bacillus subtilus* and *Pseudomonas aeruginosa,* were inoculated on the medium (Fig. 6). Under the Petri dish a grounded copper electrode was attached. In addition, an alumina plate with a powered copper electrode was placed above the medium maintaining the gap distance of 1 mm, making this like dielectric barrier discharge reactor. For this experiment, applied voltage and pulse frequency were 18 kV and 2 kHz, respectively, and duration time of plasma treatment was 1 min.

In order to confirm the chemical sterilization by neutral species during AP plasma treatment, we prepared two samples and each sample of bacteria was covered with oilpaper except plasma region to exclude neutral chemical species. Fig. 7 shows the results of these experiments. From these images we could observe the chemical effect between samples with or without oilpaper. In case of samples without oilpaper, the sterilized region was wider than the discharge region caused by reactive neutral species. So we can conclude that chemical damage by ozone, atomic oxygen, etc. is also important for plasma sterilization.

In order to analyze the relationship between sterilization results and chemical species generated in the discharge, we investigated their emission spectra using OES. For the measurement of spectra, the DBD reactor was directly mounted to the spectrophotometer with CCD detector and spectrophotometer (model ACTON research SpectroPro 300i) with a grating of 1200 grooves/mm was used. The entrance slit opening and the integration time of the CCD detector were maintained at 500 μm and 10 s, respectively.

Fig. 8 shows OES data for the pulsed DBD. The emission intensity was plotted by arbitrary unit. In Fig. 8, we observed the emission spectra of some excited atomic oxygen spectra (394.2 and 436.8 nm) and second positive system (SPS) of nitrogen including O_2^+ ionic spectra and atomic nitrogen spectra, etc. [25,26].

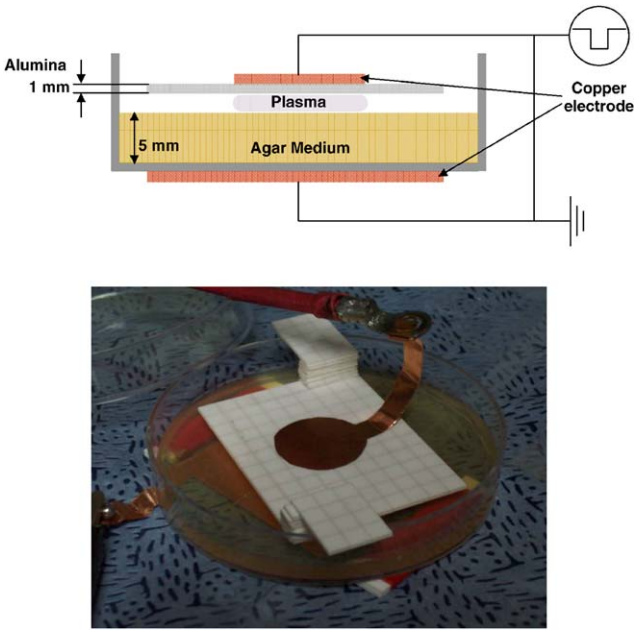

Fig. 6. Schematic diagram and image of sterilization apparatus for distinguishing between physical or chemical damage to bacteria by the plasma.

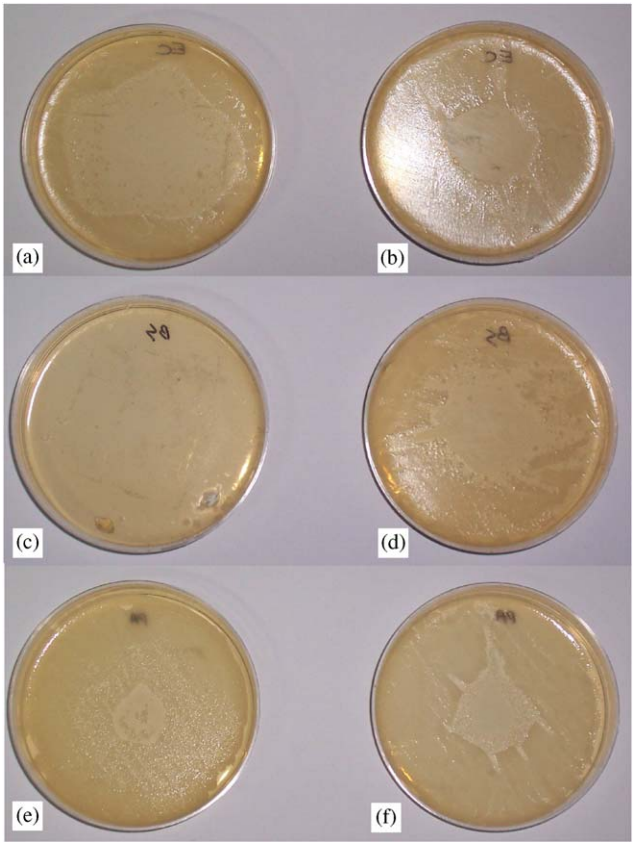

Fig. 7. Images of experimental sterilization results in case of samples with or without oilpaper. (a), (c) and (e) samples are without oilpaper. (b), (d) and (f) samples are with oilpaper. (a) and (b) for *E. coli*; (c) and (d) for *Bacillus*; (e) and (f) for *Pseudomonas*.

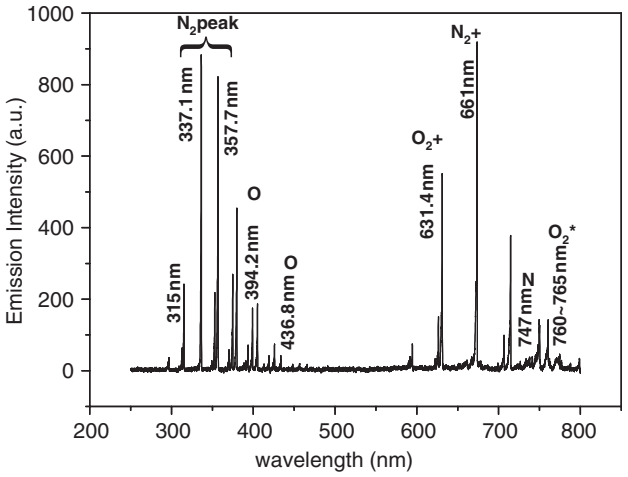

Fig. 8. OES data of pulsed DBD. Among these spectra data, the spectra of oxygen atoms (394.2 and 436.8 nm) and second positive system of nitrogen (especially 337.1 nm) are effective for sterilization.

Generally atomic oxygen is generated by a collision between an oxygen molecule and an electron [25] as

$$O_2 + e^* \rightarrow O + O + e \quad (1)$$

and ozone is generated from a three body reaction as

$$O + O_2 + M \rightarrow O_3 + M, \quad (2)$$

where M = O, O_2, or O_3 is a third collision partner.

The SPS of nitrogen, N_2^*, can also lead to the formation of oxygen atoms by the following reactions [9].

$$N_2 + e \rightarrow N_2^* + e, \quad (3)$$

$$N_2^* + O_2 \rightarrow N_2 + 2O, \quad (4)$$

$$N_2^* + O_2 \rightarrow N_2O + O, \quad (5)$$

$$O + O_2 + M \rightarrow O_3 + M. \quad (6)$$

So we can conclude that existence of atomic oxygen spectrum and SPS of nitrogen molecules means the increase of sterilization efficiency and from these results, we concluded that pulsed DBD system is very effective for sterilization.

4. Conclusion

In conclusion, we have investigated sterilization characteristics of AP plasma, especially pulsed DBD. From the sterilization results and analyses of emission spectra during AP plasma, we can conclude that both chemical damage by ozone, atomic oxygen, etc. as well as physical damage by ions in the streamer discharge are important for plasma sterilization.

Acknowledgments

This work was supported by Grant No. R01-2003-000-10476-0 from the Basic Research Program of the Korea Science & Engineering Foundation.

References

[1] E.S. Lee, H.I. Park, H.K. Baik, S.-J. Lee, K.M. Song, M.K. Hwang, C.S. Huh, Multi-jet atmospheric glow plasma cleaning of ablation debris from micro-via drilling process, Surf. Coat. Technol. 171 (2003) 307–311.

[2] E.S. Lee, H.I. Park, H.K. Baik, S.-J. Lee, K.M. Song, M.K. Hwang, C.S. Huh, Air mesh plasma for PCB de-smear process, Surf. Coat. Technol. 171 (2003) 328–332.

[3] N.-Y. Cui, N.M.D. Brown, Modification of the surface properties of a polypropylene (PP) film using an air dielectric barrier discharge plasma, Appl. Surf. Sci. 189 (2002) 31–38.

[4] F. Massines, G. Gouda, A comparison of polypropylene-surface treatment by filamentary, homogeneous and glow discharges in helium at atmospheric pressure, J. Phys. D: Appl. Phys. 31 (1998) 3411–3420.

[5] A. Grill, Cold Plasma in Materials Fabrication, IEEE Press, New York, 1994, p. 50.

[6] R. Dorai, M.J. Kushner, A model for plasma modification of polypropylene using atmospheric pressure discharges, J. Phys. D: Appl. Phys. 36 (2003) 666–685.

[7] N.K. Bibinov, A.A. Fateev, K. Wiesemann, On the influence of metastable reactions on rotational temperatures in dielectric barrier discharges in He–N_2 mixtures, J. Phys. D: Appl. Phys. 34 (2001) 1819–1826.

[8] A.S. Viner, P.A. Lawless, D.S. Ensor, L.E. Sparks, Ozone generation in DC-energized electrostatic precipitators, IEEE Trans. Ind. Appl. 28 (1992) 504–512.

[9] H.-S. Ahn, N. Hayashi, S. Ihara, C. Yamabe, Ozone generation characteristics by superimposed discharge in oxygen-fed ozonizer, Jpn. J. Appl. Phys. 42 (2003) 6578–6583.

[10] H.H. Kim, G. Prieto, K. Takashima, S. Katsura, A. Mizuno, Performance evaluation of discharge plasma process for gaseous pollutant removal, J. Electrostat. 55 (2002) 25–41.

[11] R. Hackam, H. Akiyama, Application of pulsed power for the removal of nitrogen oxides from polluted air, IEEE Electr. Insul. Mag. 17 (2001) 8–13.

[12] K. Kelly Wintenberg, A. Hodge, T.C. Montie, L. Deleanu, D. Sherman, J.R. Roth, P. Tsai, L. Wadsworth, Use of a one atmosphere uniform glow discharge plasma to kill a broad spectrum of microorganisms, J. Vac. Sci. Technol. A 17 (1999) 1539–1544.

[13] B.J. Park, D.H. Lee, J.-C. Park, I.-S. Lee, K.-Y. Lee, S.O. Hyun, M.-S. Chun, K.-H. Chung, Sterilization using a microwave-induced argon plasma system at atmospheric pressure, Phys. Plasmas 10 (2003) 4539–4544.

[14] M. Moisan, J. Barbeau, M.-C. Crevier, J. Pelletier, N. Philip, B. Saoudi, Plasma sterilization: methods mechanisms, Pure Appl. Chem. 74 (2002) 349–358.

[15] S. Lerouge, M.R. Wertheimer, L'H. Yahia, Plasma sterilization: a review of parameters, Mech. Limit. Plasmas Polym. 6 (2001) 175–188.

[16] M. Moisan, J. Barbeau, S. Moreau, J. Pelletier, M. Tabrizian, L'H. Yahia, Low-temperature sterilization using gas plasmas: a review of the experiments and an analysis of the inactivation mechanisms, Int. J. Pharmaceut. 226 (2001) 1–21.

[17] P. Koulik, S. Begounov, S. Goloviatinskii, Atmospheric plasma sterilization and deodorization of dielectric surfaces, Plasma Chem. Plasma Proc. 19 (1999) 311–326.

[18] N.S. Panikov, S. Paduraru, R. Crowe, Destruction of *Bacillus subtilis* cells using an atmospheric-pressure capillary plasma electrode discharge, IEEE Trans. Plasma Sci. 30 (2002) 1424–1428.

[19] M. Yamamoto, M. Nishioka, M. Sadakata, Sterilization by H_2O_2 droplets under corona discharge, J. Electrostat. 55 (2002) 173–187.

[20] M. Laroussi, I. Alexeff, W.L. Kang, Biological decontamination by nonthermal plasmas, IEEE Trans. Plasma Sci. 28 (2000) 184–188.

[21] T.C. Montie, K. Kelly Wintenberg, J.R. Roth, An overview of research using the one atmosphere uniform glow discharge plasma (OAUGDP) for sterilization of surfaces and materials, IEEE Trans. Plasma Sci. 28 (2000) 41–50.

[22] M. Laroussi, G.S. Sayler, B.B. Glascock, Images of biological samples undergoing sterilization by a glow discharge at atmospheric pressure, IEEE Trans. Plasma Sci. 27 (1999) 34–35.

[23] D.A. Mendis, M. Rosenberg, F. Azam, A note on the possible electrostatic disruption of bacteria, IEEE Trans. Plasma Sci. 28 (2000) 1304–1306.

[24] M. Strobel, M.C. Branch, M. Ulsh, R.S. Kapaun, S. Kirk, C.S. Lyons, Gas-phase modeling of impinging flames used for the flame surface modification of polypropylene film, J. Adhes. Sci. Technol. 10 (1996) 515–539.

[25] B. Eliasson, M. Hirth, U. Kogelschatz, Ozone synthesis from oxygen in dielectric barrier discharges, J. Phys. D:Appl. Phys. 20 (1987) 1421–1437.

[26] D.-c. Seo, T.-H. Chung, Observation of the transition of operating regions in a low-pressure inductively coupled oxygen plasma by Langmuir probe measurement and optical emission spectroscopy, J. Phys. D:Appl. Phys. 34 (2001) 2854–2861.