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Contributed Paper

Lipid Peroxidation of *Escherichia coli* by Triplet Non-thermal Atmospheric Pressure Plasma Jet

A. Hossienzadeh Colagar* [a,b], O. Alavi [a], F. Sohbatzadeh [b, c]
[a] Department of Molecular and Cell Biology, Faculty of Basic Sciences, University of Mazandaran, Babolsar, Postal Code 47416-95447, Mazandaran, Iran.
[b] Nano and Biotechnology Research Group, Faculty of Basic Sciences, University of Mazandaran, Babolsar, Postal Code 47416-95447, Mazandaran, Iran.
[c] Department of Atomic and Molecular Physics, Faculty of Basic Sciences, University of Mazandaran, Babolsar, Postal Code 47416-95447, Mazandaran, Iran.
*Author for correspondence; e-mail: acolagar@yahoo.com; ahcolagar@umz.ac.ir

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ABSTRACT

Non-thermal atmospheric plasma can be applied for sterilization of contaminated surfaces. The purpose of the present work is to study the bactericidal effects of non-thermal plasma on *Escherichia coli* and determine the level of malondialdehyde (MDA). In the present study, a non-thermal argon/air plasma jet was used to sterilize the *Escherichia coli* on solid surfaces and broth medium. The plasma jet was supplied by a sinusoidal high voltage at 18.56 kHz. Bacterial sterilization assayed by measurement of the growth inhibition zones and turbidimetry analysis in solid and broth media, respectively. The levels of MDA were measured at different time durations by thiobarbituric acid-reactive substance (TBARS) method. The study showed that increasing the exposure time leads to the extension of disinfection zone. Furthermore, MDA concentration was increased in more exposure times. It was concluded that the plasma jet could completely reduce survival of E. coli with 2×10^8 colony-forming units per ml after 10 minutes on broth medium. Such a plasma jet can be a promising device for disinfection and sterilization of bacteria in hospitals and the food industry.

Keywords: non-thermal plasma jet, *Escherichia coli*, sterilization, lipid peroxidation, MDA

1. INTRODUCTION

Sterilization is based on either a physical or a chemical process by which, all active cells, viable spores and viruses are destroyed [1,2]. It is known as a key technology in the medical industry, that is important in life quality of human beings because contaminated instruments may put the practice in jeopardy, such as fatal infection humans cause, or producing incorrect experimental results [3,4]. Autoclaves, ovens, chemicals including ethylene oxide (EtO), and radiation (gamma rays) such as conventional sterilization technologies are used in sterilization of medical instruments and implants as well as packaging materials in food industry [3,5]. These treatments are labor- and time-

consuming. Also, because of their potential damage, these aren't ideal for objects that being treated by (e.g., electronics, health-care workers' hands). Moreover, the objects that being decontaminated are exposed a severe stress: the wear of surgical instruments is signicant and implants made from thermolabile polymers lose their mechanical integrity [4,6,7]. Limitation of traditional sterilization methods has motivated the search for novel sterilization methods [8]. So we urgently need new sterilization methods having some advantages such as safety, convenience, and lack of residual toxicity [9]. Plasma-based sterilization techniques don't suffer from the traditional technique problems and they have been shown to present a great promise [10,11].

Plasma refers to an ionized medium, usually gas. The ionization degree can vary from 100% to very low values. Electrons are accelerated by electric field and ionize molecules of the working gas that pass through the discharge zone, thus this procedure create a flow of partially ionized gas or plasma. Plasma not only produces electrons and various ions, but also neutral atoms and molecules are created by it. Such as free radicals and electronically excited atoms that they have high chemical reactivity and the capability to emit UV photons [12-14]. Electrons and photons are usually known as "light" species in contrast to the other components dened as "heavy" species [15].

In man-made systems, plasma is usually generated by electrical discharges and it has classified according to its gas temperature: thermal plasmas and non-thermal (or cold) plasmas [13]. Thermal plasmas are characterized by local thermodynamic equilibrium between the electrons and the heavy species. Non-thermal plasmas are created at lower pressures and they characterized by an electron temperature that is much higher than the gas temperature, consequently they don't present a local thermodynamic equilibrium. Sterilization or inactivation by using non-thermal plasmas represents one of the promising technologies and makes the decontamination process practical, inexpensive and suitable for applications when product preservation is desired [3,15,16].

Non-thermal plasmas yield a cocktail of reactive molecules that continually react with other molecules and particles. Reactive oxygen species (ROS), reactive nitrogen species (RNS), UV irradiation, ions and electrons are emitted in the plasma discharges, which interacted with biological material or tissues [16,17]. It is well documented that ROS have profoundly damaging effects on cells through reactions with various biomacromolecules. The biological targets of ROS include DNA, proteins and lipids. Often the outer membrane is the rst and essential target for most of the chemical and/or physical decontamination techniques [15, 16, 18].

The results of our previous investigations showed that the afterglow of oxygen plasma could be efcient in the disinfection of positive and negative gram bacteria in broth and surface culture media [19,20]. The purpose of the present work, is study of the bactericidal effects of non-thermal argon/air plasma on *Escherichia coli* and determine the level of malondialdehyde in different time treatment durations, which is one of the several end products of the lipid peroxidation. Furthermore, the possible factors that may affect peroxidation levels of membrane lipids are discussed.

2. MATERIALS AND METHODS

2.1 Bacterial Strains, Media and Growth Conditions

A standard strain of *Escherichia coli* ATCC

35218 was used for this experiment. The strain was maintained as a glycerol stock cultured and stored at "70 °C until it was used. The *E. coli* stock was inoculated onto sterilized Luria-Bertani (LB) broth and grown at 37 °C for an overnight while shaken at 180 rpm. Cultured LB (0.5ml) was inoculated onto LB agar containing the following components per liter of distilled water: 10 g Bacto Tryptone; 5 g yeast extract; 10 g NaCl; and 15 g agar. The strains were maintained on standard LB agar slants at 5 °C to 6 °C. One loop from *E.coli* inoculated to 15 ml LB broth at 37 °C overnight with gentle shaking. One milliliter of previously cultured LB broth was transferred to 15 ml of fresh LB broth and incubated at 37 °C until OD_{600nm} of McFarland reached in 0.25; containing 2×10^8 colony-forming units (cfu) per ml.

2.2 Plasma Jet Source

The overall design of the experiment is shown in (Figure 1). Argon/air plasma (with 99: 1 ratio, 6 kV maximum applied voltage, 1 l/min gas flow and 18.56 kHz frequency), constituting a triple jet was used for exiting and concentrating the plasma on samples. The main structure of the plasma comprised three cylindrical electrodes which were connected to the AC source. Each of these electrodes was placed inside a glass dielectric. The working gases were employed as the medium for plasma generation. The distance between the nozzle and sample surface was kept at 25.1 mm. Furthermore, the plasma jet was 27 mm in length (Figure 1). The plasma was generated due to the occurrence of electric discharge between the two electrodes.

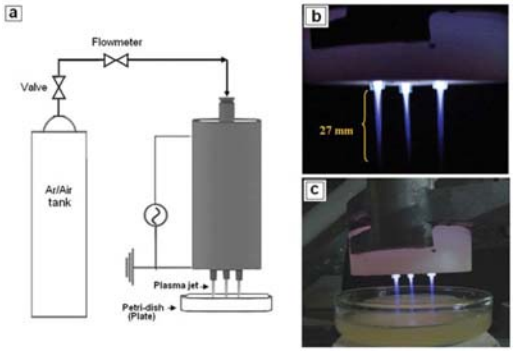

Figure 1. Arrangement of the plasma jet for bacteria sterilization: Schematic picture of the apparatus for jet discharge generation (a); A side view of the plasma jet (b); Petri-dish under the plasma device (c).

2.3 Sterilization and Bacterial Survival Assay

For surface sterilization, one milliliter of broth media with OD_{600nm}=0.25 was added to 9 ml of fresh LB broth. Afterward, 100 µl of LB containing 2×10^8 cfu.ml^{-1} bacteria spread on LB agar surface and left to dry for 20 min. Moreover, six treatments (5, 15, 30, 90, 150, 330 sec) and one control Petri dish from solid LB were prepared for this experiment. All of the samples except control were placed in front of the plasma jets. After plasma treatment, treated Petri dishes and the control Petri dish were incubated at 37 °C for overnight. Following the 16 h incubation period, the sterilization zone was clearly obvious.

For broth sterilization, 1 ml of stock suspension with OD_{600nm}= 0.25 was added in glass Petri dishes (diameter, 70 mm) containing 5ml of fresh LB medium. Four treatments (2.5, 5, 7.5 and 10 min) were

prepared for exposing with the plasma also a control Petri dish was used. The plasma exposure efficiency on bacteria was evaluated by turbidimetry analysis method.

For the turbidimetry analysis, after plasma treatment on cultured media, 1 ml of treated bacteria was added to 15ml of LB broth and incubated at 37 °C for about 2 hours (with shaking). In order to estimate the bactericidal effects of the atmospheric plasma, optical density of cultured media was measured by UV/visible spectrophotometer (UV-1600) at wavelengths ranging from 350 to 900 nm.

Also in this part is used gram staining method to investigate changes in cell Morphology after plasma exposure. The sample smears were obtained by rolling a swab into the each sample (2.5, 5, 7.5 and 10 min) and then onto a glass slide. The smears were heat fixed and Gram stained by using safranin as the counterstain. Each Gram-stained smear was evaluated for the following morphotypes under oil immersion (×1000 magnification).

2.4 Measurement of Malondialdehyde Production

Malondialdehyde (MDA) levels in liquid samples were evaluated according to the method described by Rao *et al.* 1989 and modified by Hosseinzadeh Colagar *et al.* 2009 [21,22]. MDA was assessed by using the thiobarbituric acid-reactive substance (TBARS) method. TBARS is a well-established assay for monitoring and screening lipid peroxidation. The under test sample is heated with TBA at low pH, and a pink chromogen (allegedly a $[TBA]_2$-malondialdehyde adduct) and measured by its absorbance at or close to 532 nm, or by fluorescence at 553 nm [23,24]. To determine the levels of MDA after plasma treatment on cultured media, 1 ml of the suspension was added to fresh microtube and centrifuged at 2000 g for 7 min. 100 µl of supernatant was added to 900 µl of distilled water in a glass tube. 500 µl of thiobarbituric acid reagent (0.67 g of 2-thiobarbituric acid is dissolved in 100 ml of distilled water with 0.5 g NaOH and 100 ml glacial acetic acid added) was added to the microtube and then heated in boiling water about an hour. This procedure was performed for all samples. After cooling at room temperature, microtubes were centrifuged at 4000 g for 10 min. Finally, the absorbance of the supernatant read on a spectrophotometer at 534 nm.

3. RESULTS

3.1 Surface and Liquid Sterilization

The samples were incubated at 37 °C an overnight after treatment by plasma jet to verify the ability of the plasma to sterilize. After incubating the samples, the extent of the inhibition area was obvious. The inhibition growth of bacteria, which was detected by inhibition zones, enhanced by increasing the time exposure, as can be seen in (Figure 2).

The area of the inactivated region (inhibition zone) was measured to quantify the sterilization efficiency. The extracted data of *E. coli* cultures are presented in (Table 1), that are corresponding to the approximate area of growth inhibition zones (mm^2) on agar.

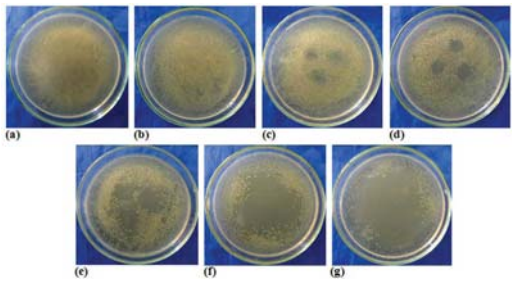

Figure 2. Appearance of growth inhibition zones on solid media in Petri dishes after exposing of atmospheric plasma jet for exposure times of: control (a); 5s (b); 15s (c); 30s (d); 90s (e); 150s (f); 330s (g).

Table 1. Area of the inhibition zones and plasma exposure time in solid surface and broth sterilization.

A) Solid surface sterilization							
Plasma exposure time	0 sec.	5 sec.	15 sec.	30 sec.	90 sec.	150 sec.	330 sec.
Inhibition zones (mm^2)	0	0	139	339	833	1568	2352
Solid inhibition zones%*	0	0	3.6	8.8	21.6	40.7	61.1
B) Broth sterilization							
Plasma exposure time	0 sec.	150 sec.	3000 sec.	450 sec.	600 sec.	-	-
Area under of turbidimetry curve**	1468	1239	1110	1056	1004	-	-
Difference between turbidimetry curve with LB***	500	270	141	87	36	-	-
Bacteria growth %****	100	54.1	28.3	17.5	7.2	-	-

*Inhibition zones%; calculated by inhibition zones per total area of the Petri dish (3846.5 mm^2) ratio.

**Measured by the area under the curve from graph in Figure 3.

***Difference between turbidimetry curve with LB; calculated by difference between areas under of theturbidimetry curve with area under of the LB turbidimetry curve (968.436).

*****Bacteria growth in liquid media%; calculated by area under of the turbidimetry curve per total area of thecontrol sample (0 min) ratio.

The spectroscopic measurement curve within 350-900 nm wavelength range (with UV-1600 UV/visible spectrophotometer) is shown in (Figure 3). On the sterilization in LB broth, the curve of the control sample (0 min) was higher than the others. Furthermore, the LB curve located at the bottom of the others. The curves related to the exposure time of 2.5, 5, 7.5 and 10 min are placed after the control (0 min) curve. In (Table 1), area under the turbidimetry curve and plasma exposure time was presented. Area under the turbidimetry curve represents the number of bacteria that grown in the LB broth. Consequently further growth increased Area under of the turbidimetry curve. Microscopic picture from gram staining of *E. coli* samples is shown in (Figure 4). According to that, this is obvious that, more change in the cell membrane of *E. coli* is created by enhance in the exposure time of plasma.

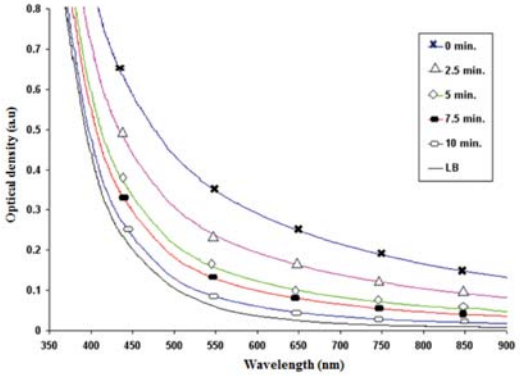

Figure 3. Turbidimetry measurement curves for reduction of survived bacteria in LB broth: four treatments (2.5, 5, 7.5 and 10 min.) compared by control sample (0 min.) from liquid medium; LB is positive control and LB curve indicated that culture media is without other contaminates.

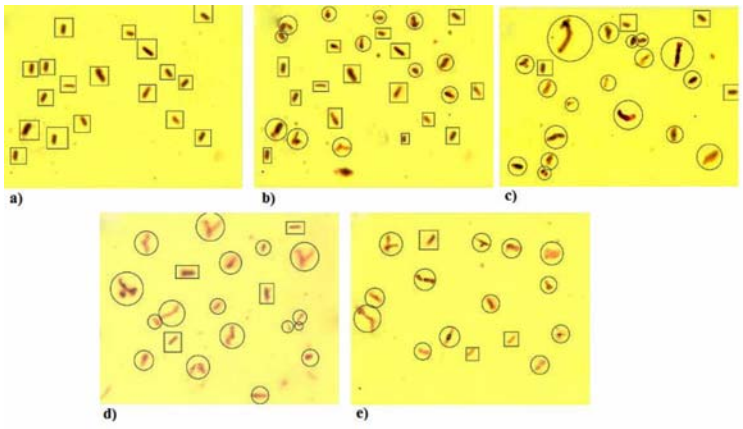

Figure 4. Gram-stained gram negative *E. coli* smears from exposured plasma samples: control (a); 2.5 min. (b); 5 min (c); 7.5 min. (d) 10 min. (e) ; Normal *E. coli* cells marked by the square (□) and the *E. coli* cells that have been changed by plasma effects marked by the circle (O).

3.2 The Changes of MDA Concentration

The bombardments of *E.coli* by the non-thermal plasma can cause damaging action on cell surface. This process can cause lipid peroxidation of bacterial outer membrane and leads to the formation of MDA. After the plasma exposure, changes in the malondialdehyde concentration can reflect the interactions between oxygen radicals produced by plasma and bacterial cell. (Figure 5) represents the changes of MDA concentration during different times of exposure.

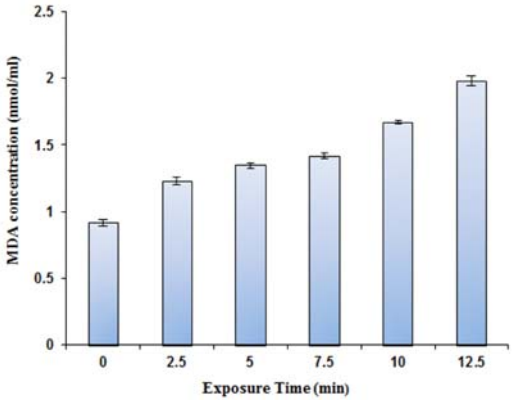

Figure 5. Correlation of the MDA Levels with exposure time bombardment.

4. DISCUSSION

4.1 Surface and Liquid Sterilization

The obtained results from the bombardment of solid surfaces by argon/air plasma clearly indicated that the non-thermal plasma can effectively sterilize the surfaces of materials. Two areas on the surface of agar were obviously distinct from each other on the treated Petri dishes after incubation. Areas, where bacteria were inactivated, looked like intact agar (round transparent zones) and marked the growth inhibition, whereas areas that were changed in color and appearance showing the bacteria grow zone. Furthermore, there was an area with a lower bacterial density, between these two regions. In fact, there were three areas after treatment with plasma: complete inhibition zone, partial inhibition zone and non-affected zone. The growth inhibition zone do not found for 5 s plasma exposure in *E. coli*. The area of inhibition zones for 15, 30, 90, 150 and 330 s plasma exposure were 139, 339, 833, 1568 and 2352 mm^2, respectively. It should be emphasized that the inhibition zone is not confined to the diameter of the plasma jet. It was seen that the area of inhibition zone extends when the exposure

time increases. Therefore, regarding to these results, the lethal effects that revealed in the *E. coli* cultures are due mainly to the molecular damages caused by exiting components from plasma jet, such as reactive species and electrons, whereas the UV-generated ozone could also have some remarkable contribution to the biomolecules oxidation [19,25].

According to (Figure 3), it is obvious that the area under the curve is reduced when the exposure time of plasma increased on LB broth. A significant reduction in the amount of bacteria was observed at 2.5 min exposure time. After 5 min, more than 70 percent of the bacteria were destroyed and there was a significant decrease in the area under the curve. It was seen that the plasma exposure completely reduced survival of *E. coli* after 10 min. A significant difference of survival could be evaluated by comparing the control and 10-min treated samples. We applied a fast and dependable turbidimetric method to detection of bacterial decontamination after plasma exposure.

This study proposes that the plasma jet is an effective method to disinfect *Escherichia coli* from certain environments. These environments could include hospitals; operating rooms; food factories and so on. For example, disinfection by non-thermal plasma jets can be a promising method to eliminate bacteria in the food industry. Foodborne diseases are caused by *Campylobacter*, *Salmonella*, *Listeria monocytogenes*, and *E. coli* O157:H7 take a major crisis on health. Note that conventional methods of killing pathogens in foods have relied on heating and it causes unwanted side-effects on the sensory, nutritional, and functional properties of food [26,27]. Therefore, plasma-based sterilization techniques can replace customary methods of killing pathogens in the food industry.

However, it should be noted that the bacterial growth in the medium is very different than that in the environment. For example, adequate nutrients can be found for bacteria in LB medium. These conditions and the optimal temperature of 37 °C lead to bacterial growth in logarithmic phase. Furthermore, the damaged bacteria can repair their failure and grow. It is obvious that such a situation does not exist in the environment. Therefore, we expect that the number of surviving bacteria in the environment may be less than that in the medium and the non-thermal plasma could be more effective in killing bacteria.

In fact, plasma inactivation may be induced by reactive species, electrons, positive and negative ions and ultraviolet (UV) photons. All or most of these species are capable of causing oxidative damage to bacteria, and if the damage was extensive, it will lead to cell death. In atmospheric pressure plasma most of the UV radiation is absorbed by air molecules and could not be delivered to the treated surface. ROS such as hydroxyl radical (OH), superoxide anion radical ($^{\bullet}O_2^-$) and singlet molecular oxygen (O_2 ($^1\Delta_g$)) can cause a great amount of damage to macromolecules. Ozone and ROS lead to break induction in DNA strands and specic formation of 8-oxoguanine or increased activity of poly (ADP-ribose) synthetase [19, 24]. Moreover, $OH^{\bullet}$ can attack proteins and lipids in cell membrane and interfere with intracellular materials such as DNA, and thus is bactericidal. It can oxidize almost everything, especially reacts with unsaturated fatty acid on the cell membrane, causing membrane damage and cell rupture [28,29].

4.2 The Changes of MDA Concentration

The results showed that, the MDA concentration increased gradually by increasing the exposure time. High concentrations of free radicals can cause oxidation of unsaturated fatty acids. The MDA is produced by the plasma treatment, can be one of the leading causes of death in bacteria. In principle, consequently, the content of unsaturated fatty acids in the bacterial outer membrane is reduced by increasing MDA concentrations [11].

Peroxidation of phospholipid bilayer is known as a cause of cellular death, through a chain process that leads to the formation of DNA adducts. The result of lipid peroxidation process is formation of MDA. In *Salmonella typhimurium*, MDA induces insertions and deletions as well as base substitutions. Therefore, MDA is a potentially important contributor to DNA damage and mutation [30-32].

As mentioned earlier, non-thermal plasma can generate a mixture of radicals and reactive species. By radical attack to the unsaturated fatty acids of cell membrane, initiate lipid peroxidation (Figure 6). The lipid peroxidation chain reaction is initiated by ROS (or any reactive species) attack to the unsaturated fatty acid and abstracted H atom from a methylene group (-CH2). This reaction leads to formation of a fatty-acid radical ($L^{\bullet}$). It can readily react with an oxygen molecule to give a lipid peroxyl radical ($LOO^{\bullet}$). These radicals can abstract H atoms from other lipid molecules (LH) to become LOOH. $LOO^{\bullet}$ is unstable and breaks down to form various products such as aldehydes (malondialdehyde and 4-hydroxy-nonenal) [16,19,33].

The results of this study indicated that the non-thermal atmospheric pressure plasma jet can effectively sterilize solid and liquid surfaces. Reactive species such as singlet oxygen, ozone, and hydroxyl radicals could be involved in this process. These species, along with other radicals could participate in the oxidation of unsaturated lipids in bacterial membranes and the production of MDA. Therefore, MDA production and membrane damage can be an important factor in rapid death of bacteria.

$$LH + X^{\bullet} \rightarrow L^{\bullet} + XH$$
$$L^{\bullet} + O2 \rightarrow LOO^{\bullet}$$
$$LOO^{\bullet} + LH \rightarrow L^{\bullet} + LOOH$$
$$LOO^{\bullet} \rightarrow \text{MDA and nonenal}$$

Figure 6. Basic reaction sequence for the process of lipid peroxidation by free radicals.

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REFERENCES

[1] Kelly Wintenberg K., Hodge A. and Montie TC., *J. Vacuum Sci. Technol.,* 1999; **17**: 1539-1544. DOI 10.1116/1.581849.

[2] Lee K.W., Park B.J., Lee D.H., Lee I.S., Hyun S.O., Chung K.H. and Park J.C., *Surf. Coat. Technol,* 2005; **193**: 35-38. DOI 10.1016/j.surfcoat.2004.07.034.

[3] Chiang M.H., Wu J.Y., Li Y.H., Wu J.S., Chen S.H. and Chang C.L., *Surf. Coat. Technol,* 2010; **204**: 3729-3737. DOI 10.1016/j.surfcoat.2010.04.057.

[4] Opretzka J., Benedikt J., Awakowicz P., Wunderlich J. and von Keudell A., *J. Phys. D: Appl. Phys.,* 2007; **40**: 2826-2830. DOI 10.1088/0022-3727/40/9/024.

[5] Schneider J., Baumg rtner K.M., Feichtinger J., Kr ger J., Muranyi P.,

Schulz A., Walker M., Wunderlich J. and Schumacher U., *Surf. Coat. Technol.*, 2005; **200**: 962-966. DOI 10.1016/j.surfcoat.2005.01.114.

[6] Akishev Y., Grushin M., Karalnik V., Trushkin N., Kholodenko V., Chugunov V., Kobzev E., Zhirkova N., Irkhina I. and Kireev G., *Pure Appl. Chem.,* 2008; **80**: 1953-1969. DOI 10.1351/pac20088 0091953.

[7] Burts ML., Alexeff I., Meek E.T. and McCullers J.A., *Am. J. Infect. Control.,* 2009; **37**: 729-733. DOI 10.1016/j.ajic. 2009.03.010.

[8] Ohkawa H., Akitsu T., Tsuji M., Kimura H., Kogoma M. and Fukushima K., *Surf. Coat. Technol,* 2006; **200**: 5829-5835. DOI 10.1016/j.surfcoat.2005.08.124.

[9] Yang L., Chen J. and Gao J., *J. Electrost.,* 2009; **67**: 646-651. DOI 10.1016/j.elstat. 2009.01.060.

[10] Laroussi M., Leipold F., *Int. J. Mass Spect.,* 2004; **233**: 81-86. DOI 10.1016/j.ijms. 2003.11.016.

[11] Liu H., Chen J., Yang L. and Zhou Y., *Appl. Surf. Sci.,* 2008; **254**: 1815-1821. DOI 10.1016/j.apsusc.2007.07.152.

[12] Bogaerts A., Neyts E., Gijbels R. and van der Mullen J., *Atomic Spect.,* 2002; **57**: 609-658. DOI 10.1016/S0584-8547(01) 00406-2.

[13] Kalghatgi S., Kelly C., Cerchar E., Torabi B., Alekseev O., Fridman A., Friedman G. and Azizkhan-Clifford J., *PLoS ONE.,* 2011; **6**: 16270. DOI 10.1371/journal.pone. 0016270.

[14] Venezia R.A., Orrico M., Houston E., Yin S.M. and Naumova Y.Y., *Infect. Control Hosp. Epidemiol.,* 2008; **29**: 430-436. DOI 10.1086/588003.

[15] Moreau M., Orange N. and Feuilloley M.G.J., *Biotechnol. Adv.,* 2008; **26**: 610-617. DOI 10.1016/j.biotechadv.2008.08.001.

[16] Gaunt L.F., Beggs C.B. and Georghiou G.E., *IEEE T. Plasma Sci.*, 2006; **34**: 257-1269. DOI 10.1109/TPS.2006. 878381.

[17] Rupf S., Lehmann A., Hannig M., Schafer B., Schubert A., Feldmann U. and Schindler A., *J. Med. Microbiol.*, 2010; **59**: 206-212. DOI 10.1099/jmm.0. 013714-0.

[18] Cabiscol E., Tamarit J. and Ros J., *Int. Microbiol.,* 2000; **3**: 3-8. PMID 10963327.

[19] Hosseinzadeh Colagar A., Sohbatzadeh F., Mirzanejhad S. and Valinataj Omran A., *Biochem. Eng. J.,* 2010; **51**: 189-193. DOI 10.1016/j.bej.2010.06.017.

[20] Sohbatzadeh F., Hosseinzadeh Colagar A., Mirzanejhad S. and Mahmodi S., *Appl. Biochem. Biotechnol.,* 2010; **160**: 1978-1984. DOI 10.1007/s12010-009-8817-3.

[21] Hosseinzadeh Colagar A., Pouramir M., Tahmasbpour Marzony E. and Jorsaraei S.G.A., *Braz. Arch. Biol. Technol.,* 2009; **52**: 387-1392. DOI 10.1590/S1516-8913200 9000600010.

[22] Rao B., Souflir J.C., Martin M. and David G., *Gamete Res.*, 1989; **24**: 127-34. DOI 10.1002/mrd.1120240202.

[23] Halliwell B., Chirico S., *Am. J. Clin. Nut.,* 1993; **57**: 715-725.

[24] Joshi S.G., Cooper M., Yost A., Paff M., Ercan U.K., Fridman G., Friedman G., Fridman A. and Brooks A.D., *Am. Soc. Microbiol.,* 2011; **55**: 1053-1062. DOI 10.1128/AAC.01002-10.

[25] Ramona F., Antoniea P., Iuliana M., Andrei N., Dorina C. and Gheorghe P., *Afr. J. Biotechnol.,* 2012; **11**: 4234-4240. DOI 10.5897/AJB11.3783.

[26] Song H.P., Kim B., Choe J.H., Jung S., Moon S.Y., Choe Y. and Jo C., *Food Microbiol.,* 2009; **26**: 432-436. DOI 10.1016/j.fm.2009. 02.010.

[27] Velusamy V., Arshak K., Korostynska O., Oliwa K. and Adley K., *Biotechnol. Adv.,* 2010; **28**: 232-254. DOI 10.1016/j.biotechadv. 2009.12.004.

[28] Sun P., Wu H., Zhu W., Zhang J. and Fang J., *Plasma Process Polym.,* 2012; **9**: 157-164. DOI 10.1002/ppap.201100041.

[29] Wu H., Sun P., Feng H., Zhou H., Wang R., Liang Y., Lu J., Zhu W., Zhang J. and Fang J., *Plasma Process Polym.,* 2012; **9**: 417-424. DOI 10.1002/ppap. 201100065.

[30] Dobrynin D., Fridman G., Friedman G. and Fridman A., *New J. Phys.,* 2009; **11**: 115020. DOI 10.1088/1367-2630/11/11/115020.

[31] Dubovskiy I.M., Martemyanov V.V., Vorontsova Y.L., Rantala M.J., Gryzanova E.V. and Glupov V.V., *Comparative Biochem. Physiol. Part C*, 2008; **148**: 1-5. DOI 10.1016/j.cbpc.2008.02. 003.

[32] Niedernhofer L.J., Daniels J.S., Rouzer C.A., Greene R.E. and Marnett L.J., *J. Chem. Biol.,* 2003; **278**: 31426-31433. DOI 10.1074/jbc.M212549200.

[33] Devasagayam T.P.A., Boloor K.K. and Ramasarma T., *Indian J. Biochem. Biophys.,* 2003; **40**: 300-308.

[34] Gweon B., Kim D.B., Moon S.Y. and Choe W., *Curr. Appl. Phys.*, 2009; **9**: 625-628. DOI 10.1016/j.cap.2008.06. 001.

[35] Ragni L., Berardinelli A., Vannini L., Montanari C., Sirri F., Guerzoni M.E. and Guarnieri A., *J. Food Eng.*, 2010; **100**: 125-132. DOI 10.1016/j.jfoodeng.2010. 03.036.