Romanian Reports in Physics, Vol. 68, No. 3, P. 1197–1207, 2016

STUDY ON UV-VISIBLE EMISSION PLASMAS WITH APPLICATIONS IN PHOTODYNAMIC THERAPY AND SURFACE TREATMENT AGAINST BIOLOGICAL CONTAMINANTS

O.G. POMPILIAN, P. DINCA, C. POROSNICU*, C.P. LUNGU, P. CHIRU, B. BUTOI, I. JEPU

National Institute for Laser, Plasma and Radiation Physics INFLPR, 409 Atomistilor Str., 077125 Magurele, Romania
*Corresponding author: corneliu.porosnicu@inflpr.ro

Received March 11, 2015

Abstract. In the last decade, the uses of plasma in medical applications have shown positive results thus increasing the interest in this field. For a drug delivery system capable of accurate dosing of active substances inside tumors and pathogen cells, light sensitive photoactive colorants are used. The photodynamic therapy has proved to be an efficient method in treating bacteria and fungus. In this paper, we present two plasma devices designs built for investigating the possibility of using them in decontamination. Time depending electrical characterization as well as spectra analysis was performed for different concentrations of Xe and Ar gas flow as well as temperature investigation to ensure a non-thermal treatment. After obtaining the concentration for best spectral emission, the atmospheric pressure pulsed plasma was used in treating several microorganisms with promising results.

Key words: photodynamic therapy, plasma jet.

1. INTRODUCTION

Photodynamic therapy is a method used for pathogen cells and tumors inactivation using photoactive colorants, sensible to light and a specific wavelength [1]. The energy transfer from the photoactive colorant to oxygen leads to the formation of extremely toxic oxygen species as free radicals and $^1O_2$ singlet oxygen, which deactivates the pathogen agents [1]. The photodynamic therapy is an extremely selective method [2] concerning the pathogen cells due to photoactive colorants ability to identify and conglomerate them. This therapy was successfully used to treat several diseases like abnormal cellular growth as cancer, rheumatoid arthritis, vitiligo, pathologic myopia, macular degeneration caused by age and arteriosclerosis. The greatest step forward was in cancer treatment but the photodynamic therapy showed great efficiency in treating the surfaces contaminated with biologic contaminants (bacteria, fungus etc.) [1–5].

The photodynamic therapy is based on the interaction between visible light and a photo sensible agent, which by luminous activation generates short life species of cytotoxic agents. Under excitation effect, the photo sensible agent is converted from singlet state in triplet state by a system that reacts with molecules around it to produce radical species and hydrogen peroxide – known as first type mechanism or it transfers the energy to molecular oxygen $(^3O_2)$ to produce singlet oxygen $(^1O_2)$ – known as second type mechanism. Both mechanisms have as result the cell localized death; however, the main photodynamic mechanism seems to be the second type, which generates singlet oxygen, extremely toxic [6]. From clinical point of view, once the photosensibilisation medicine reaches the maximum level within the target tissue, a dose of light carefully adjusted is applied to activate the compound and the photodynamic attack starts. For superficial affections of the skin, the light is applied directly on the affected tissue while for internal affections the light source is connected to a catheter with optical fiber, which allows reaching the targeted organ. Usually, high laser sources are used, but their high costs make inaccessible the photodynamic treatment for many institutions and thus the dissemination of this technology is difficult within many countries. Recently, many incoherent alternative light sources have been analyzed to replace the laser within the photodynamic treatment. Between these, we decided to study the discharge devices in plasma produced in a 10–10^5 Pa pressure range with emission in UV-visible range [5–10].

2. EXPERIMENTAL DEVICES

Within this phase, two experimental devices were used: (i) a quartz tube with coaxial electrodes, where the generated luminous radiation is exteriorly oriented (Fig. 1), within a variable frequency pulsed discharge. It was designed an experimental system for the spectral and electric study of bright emission produced as a result of pure and mixed gases low atmospheric pressure discharge.

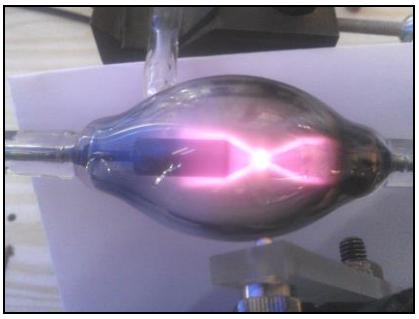

Fig. 1 – Experimental device for the study of bright radiation produced within high-pulsed voltage discharge.

The experimental system contains a discharge tube made of a quartz balloon provided with two metallic electrodes, connected to a vacuum system provided with turbo molecular pump (limit vacuum in the range 10^{-7} torr), an admission and mixing pure gas system, a high voltage and variable frequency external generator. We used high purity (≥ 99.998%) plasmagen gases, Ar and Xe. The usual voltage source used is a radiofrequency source with the following parameters: electric voltage (1–2kV) and frequency (120–400) kHz [11–17]. The emitted spectra were recorded by capturing the optic signals with a quartz lens with focal distance of 80 mm and an optical fiber to transmit the light to an optical multichannel analyzer (OMA) having a spectral range of 200–900 nm and 0.5 nm resolution. The recording time used was of 500 ms. The electric signals were recorded with a digital oscilloscope and a digital multimeter. The schematic draw of the experimental setup including the device which uses plasmagen gas at atmospheric pressure is shown in Fig. 2. This is made of a tube containing the gas system and the cathode. The circular anode is a metallic disc shaped with a central hole of 1 mm, insulated against the cathode through a circular part made of Teflon. The distance between these two electrodes is adjustable (1–10 mm). Working gas admission is released near the discharge area.

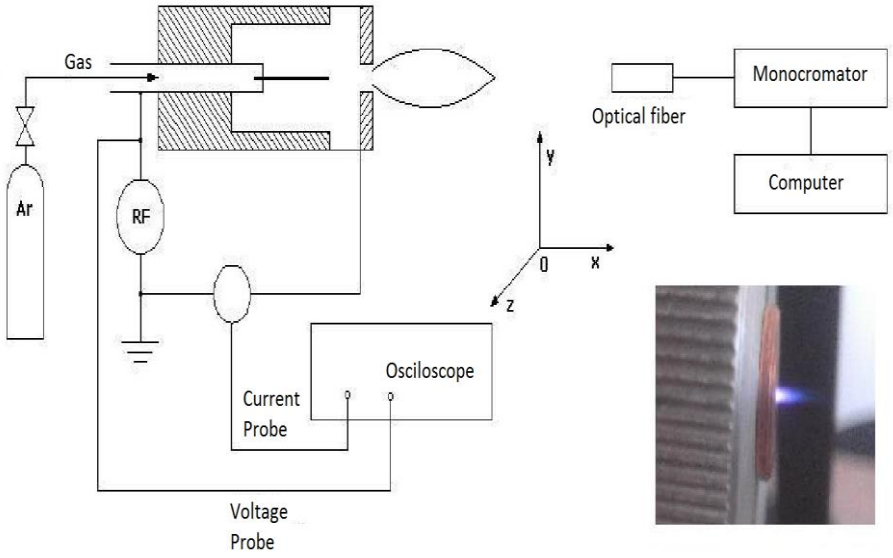

Fig. 2 – Experimental device diagram for the study of gas discharges at atmospheric pressure.

The electric supply from a pulsed voltage source is realized by a device which allows voltage and current probes coupling to be used for electric measurements. The gas is constrained to flow through the interelectrodic space and

to be released through the electrode grounded nozzle. Thus, the plasma ignited in the discharge between the two electrodes expands through the nozzle to the exterior.

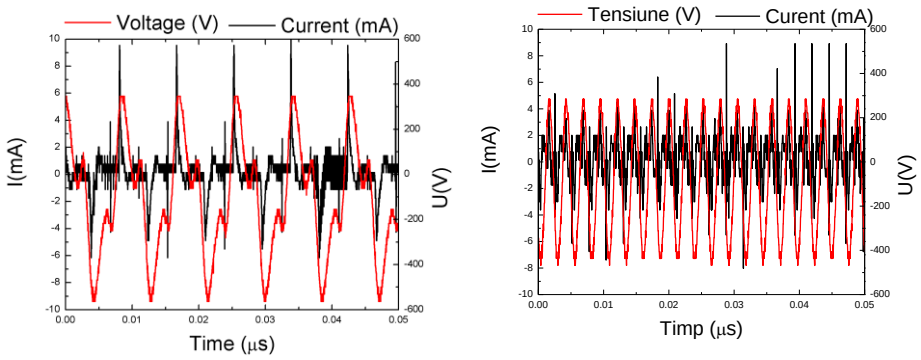

Fig. 3 – Temporal evolution of current and voltage for plasma ignited in argon gas at 4 torr pressure at two working frequencies: 9.7 kHz (left) and 31.7 kHz (right).

The experimental system contains an image capturing thermovision camera to measure the device cathode temperature and a spectroscopic chain to record the optical emission produced by plasma. The wave shapes for current and voltage recorded using a digital oscilloscope and are shown in Figs. 3–5. Figure 3 and the detailed Fig. 4 show that at high frequency (~31 kHz) the voltage signal generated by the supply is perfectly sinusoidal, while for the lowest frequency, even though the supply generates a sinusoid, the discharge forces the voltage signal to an irregular variation. Due to this fact, the maximum values of the discharge current were obtained when the frequencies were between 9 and 32 kHz.

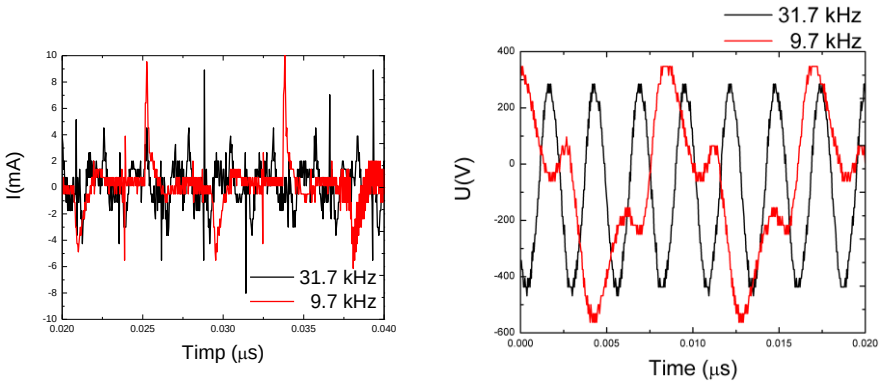

Fig. 4 – Current (left) and voltage (right) characteristics at maximum and minimum frequencies respectively, for argon plasma at working pressure of 4 torr.

For different working pressure values, even though the voltage remains unchanged, the maximum value of the current drops dramatically with working pressure value increase (Fig. 5 upper part). This discharge current varies also with the Ar/ Xe relative concentrations ratio (Figs. 6 and 7).

Thus, even though there is a maximum value for discharge current of pure argon and an obvious drop of its value until Xe 20%–Ar 80% concentration, after that there is no obvious variation trend observed.

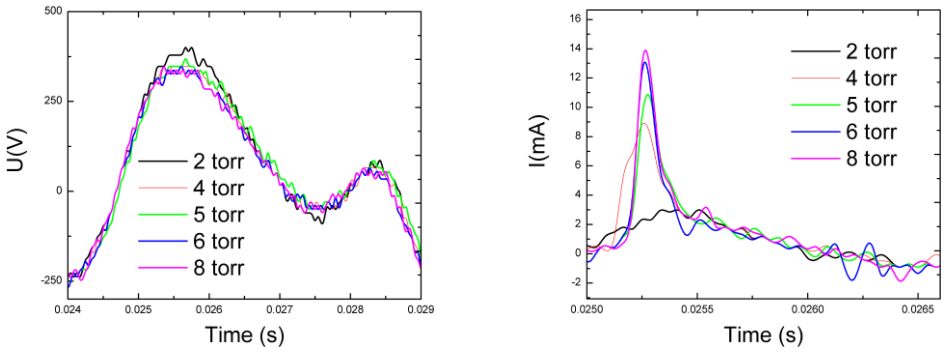

Fig. 5 – The discharge voltage (left) and current (right) respectively for different pressures of Ar at 9.7 kHz frequency.

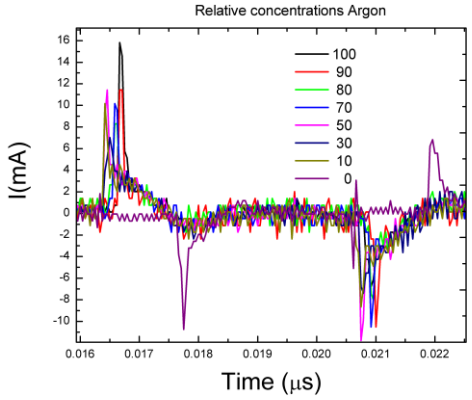

Fig. 6 – The discharge current for different Ar-Xe concentrations at 9.7 kHz frequency.

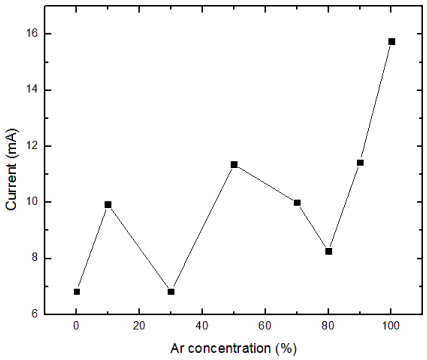

Fig. 7 – Maximum value of discharge currents for different Ar-Xe concentrations at 9.7 kHz frequency.

3. RESULTS

3.1. PULSED DISCHARGES OBTAINED IN SEALED DISCHARGE TUBES

Using the experimental system presented in section 2 (i) the spectral emission was studied according to the used frequency for igniting and maintaining the discharge in pure or mixed gases. In Figs. 8 and 9, the typical optical spectral emission for Ar and Xe respectively discharges are presented and the identification of optical emission lines. The spectral lines were identified for the excited atoms of Ar and Xe. The spectral lines were identified by comparison with NIST database. (www.nist.gov).

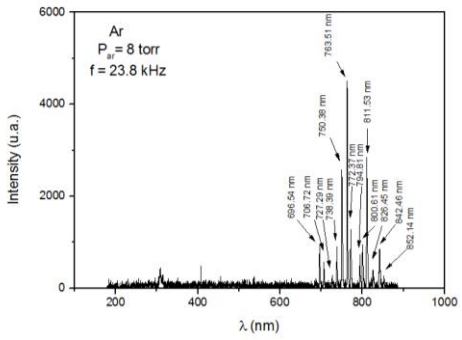

Fig. 8 – Typical emission spectrum in Ar; P = 8 torr.

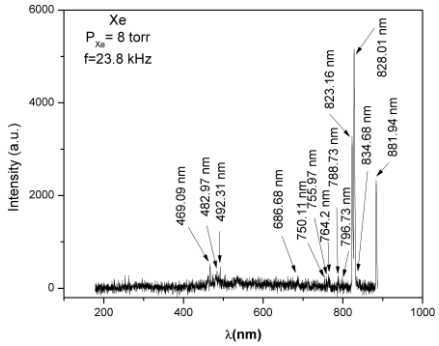

Fig. 9 – Typical emission spectrum in Xe; P = 8 torr.

From the measurement of discharge radiation intensity in xenon, for 2 working pressure, of 2 and 8 torr respectively, dependencies on the applied frequency were obtained (Figs. 10–11).

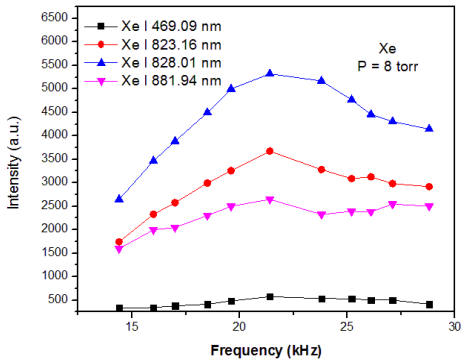

Fig. 10 – The dependency of radiation intensity in Xe discharge at 8 torr pressure depending on the applied frequency.

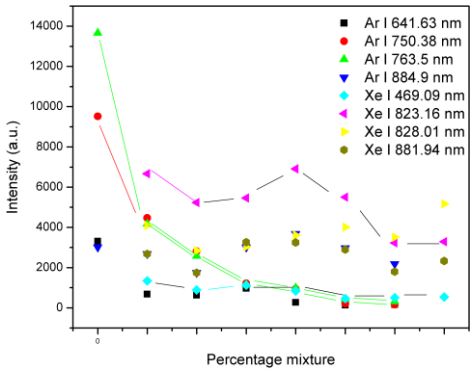

Fig. 11 – Dependency on Ar and Xe emission intensities from Ar:Xe mixture depending on the concentration of the two gases.

As shown in Fig. 10 it was observed that the intensities of the spectral emission lines present maximum values when the supply frequency is in the range 20–25 kHz, in conditions of identical values of gas pressure and applied voltage. As a result, to perform the experiments, the frequency value of 23.8 kHz was chosen. The evolution of spectral lines intensities depending on the relative concentration of the two used gases: Ar and Xe is also shown. There have been studied the spectral characteristics of the discharges obtained in working gas with total pressure of 8 torr: 100%Ar/0%Xe, 90%Ar/10%Xe, 80%Ar/20%Xe, 70%Ar/30%Xe, 50%Ar/50%Xe, 30%Ar/70%Xe, 20%Ar/80%Xe, 10%Ar/90%Xe, 0%Ar/100%Xe. The spectral lines corresponding to transitions with wavelength Ar I 750.38 nm and Ar I 763.5 decrease in intensity proportionally with the drop of Ar concentration in the mixture while Ar I 884.9 nm and Ar I 641.63 nm spectral lines do not follow this trend. Also, the Xe I 823.16 nm and Xe I 469.09 nm emission lines have increased intensities when the working gas consists in a 50%Ar: 50%Xe mixture, proving the production of several excitation and preferential emission transfers of several wavelengths that can be selected for photodynamic treatment.

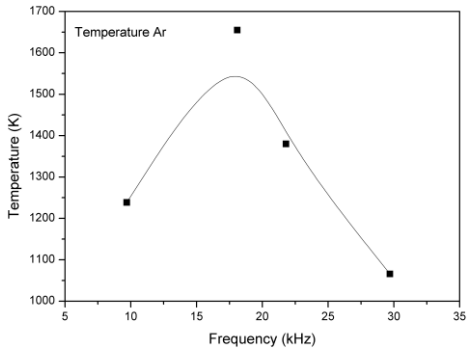

Fig. 12 – Electron temperature dependency on the applied frequency.

Taking into consideration that the local thermodynamic equilibrium and using Boltzmann plotting method [18, 19], the electron temperature dependency was determined depending on the applied frequency. In Fig. 12 it is observed the variation between 1000 and 1700 K the maximum recorded at a frequency of ~18 kHz with its sharp decrease, at 9.7 kHz the temperature is about 1000 K.

3.2. PULSED DISCHARGES OBTAINED IN PLASMA JET AT ATMOSPHERIC PRESSURE

Using the device presented in Fig. 2, the emission spectra were collected, and the diagram is shown in Fig. 13. The Gaussian distribution of spectral lines intensities can be observed and especially the spectral emission corresponding to OH molecule, responsible with the production of the active species with crucial role in contaminants treatment. The working gas was argon; OH radical was obtained due to plasma jet interaction with the air [20, 21].

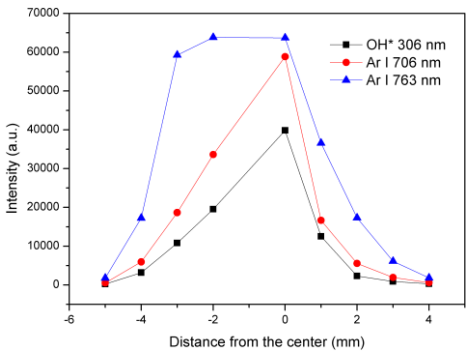

Fig. 13 – Diametral distribution of OH and Ar line intensities.

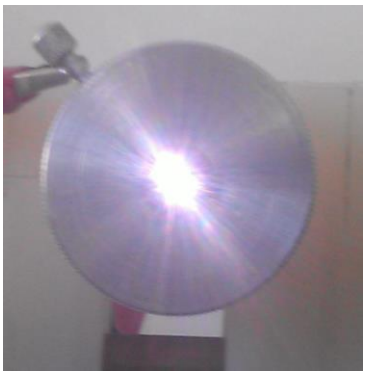

Fig. 14 – Plasma jet image at the outer part of the device.

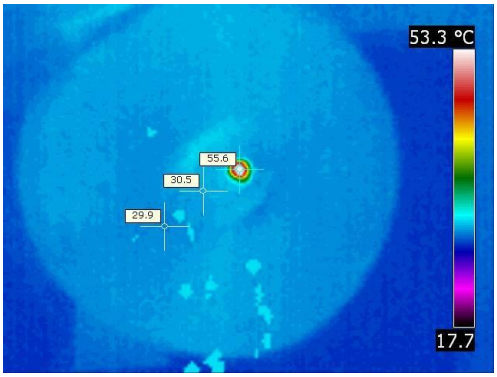

Fig. 15 – Plasma jet temperature measured using a thermovision camera.

A mini plasma-torch working at atmospheric pressure was designed and tested, presented in detail in Fig. 16. Plasma jet pattern and temperature (do not exceed 54^{o}C) at the outer part of the device are presented in Figs. 14 and 15.

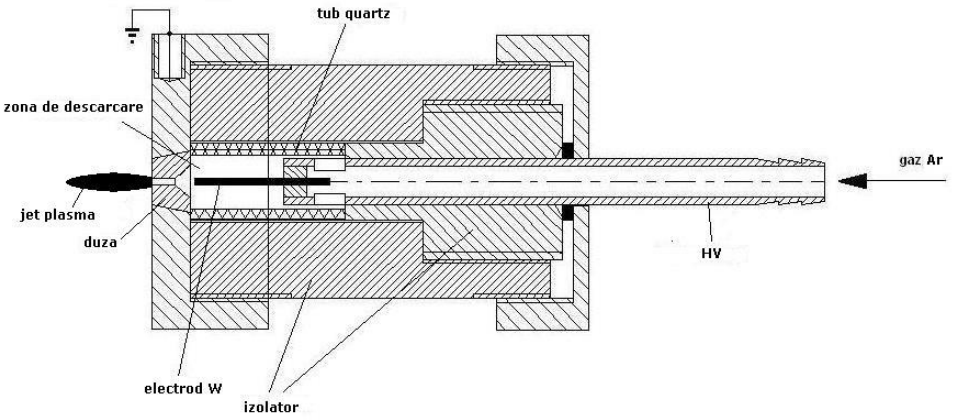

Fig. 16 – Detailed diagram of the mini plasma-torch working at atmospheric pressure.

3.3. TESTS REGARDING PULSED NON-THERMAL PLASMA JETS AT ATMOSPHERIC PRESSURE ON SEVERAL MICROORGANISMS

Within the research collaboration between ICCF and our institute, a working algorithm was developed through which the physical parameters of plasma flux were decided as well as the microbe strains (bacteria and fungus) on which they were applied. The following microbe strains were used: *Escherichia coli* (ATCC 8739), *Staphylococcus aureus* (ATCC 6538) and *Candida albicans* (ATCC 10231). To prepare these cultures we needed to retort starting from the stock culture, in solid environment, B environment (Triptona Soya Agar) pre-poured leant and for 48 h the fungus culture was incubated at 25^{o}C. Three plates were selected (one for each working culture type), and on each plate three working areas were delimited, numbered 1, 2, 3. All three cultures were restrictedly exposed to a plasma jet (Jet 1) in the working area number 1 on the plate ($Ø$ = 10 mm), directly on the cellular culture surface, using parallel and perpendicular moves in horizontal plane, at 1 cm distance for 1 minute. After the direct exposure procedure – in an area of 10 mm diameter – on the surface of the culture in each Petri bowl, at room temperature, they were sealed and incubated for 24 hour at 30–35 ^{o}C for bacteria and 20–25 ^{o}C for fungus. The obtained results showed modifications of microorganisms' growth (apoptosis) for those subjected to the study, in the tested working variants.

4. CONCLUSIONS

Within the present phase, there have been developed two discharge devices for the plasma production in a pressure range of 10–10^5 Pa with UV-visible emission for applications in photodynamic therapy and treatment of surfaces against biologic contaminants. Were developed sealed tubes and plasma jet devices working under and respectively at atmospheric pressure. There have been characterized the plasmas ignited in that devices to obtain radiations of interest for photodynamic therapy and treatments against biologic contaminants. A maximum electron temperature of 1700 K at ~18 kHz frequency was estimated using the emitted radiation of the sealed tube filled with 50%Ar: 50%Xe mixture. A mini plasma-torch prototype working at atmospheric pressure was developed and applied for treatment of some microbe strains as *Escherichia coli* (ATCC 8739), *Staphylococcus aureus* (ATCC 6538) and *Candida albicans* (ATCC 10231). It was demonstrated modifications of microorganisms' growth (apoptosis) for those cells subjected to the study.

Acknowledgements. This work was supported by a grant of the Romanian National Authority for Scientific Research, CNDI– UEFISCDI; project number 80/2014, PN-II-PT-PCCA-2013-4-2165.

REFERENCES

1. N. Philip, B. Saoudi, M.-C. Crevier, M. Moisan, J. Barbeau, J. Pelletier, *The respective roles of UV photons and oxygen atoms in plasma sterilization at reduced gas pressure: the case of N2-O2 mixtures*, IEEE Transactions on Plasma Science **30**, 1429–1436 (2002).
2. Vyacheslav V. Tsiolko, *Features of the Sterilization by VUV/UV Irradiation of Low-Pressure Discharge Plasma*, in: *Plasma for Bio-Decontamiantio, Medicine and Food Security*, Springer, 2012, pp. 231–249.
3. K. Lee, K.H. Paek, W.T. Ju, Y. Lee, *Sterilization of bacteria, yeast, and bacterial endospores by atmospheric-pressure cold plasma using helium and oxygen*, Journal of Microbiology **44**, 269–275 (2006).
4. M. Moisan, B. Saoudi, J. Pelletier, J. Barbeau, *Understanding the respective roles of UV photons and radicals in cold plasma sterilization*, IEEE International Conference on Plasma Science ICOPS 2002 (2002).
5. J. Pollak, M. Moisan, D. Keroack, M. K. Boudam, *Low-temperature low-damage sterilization based on UV radiation through plasma immersion*, Journal of Physics D: Applied Physics **41**, 135212 (2008).
6. S. Lerouge, M. R. Wertheimer, L'H. Yahia, *Plasma Sterilization: A Review of Parameters, Mechanisms, and Limitations*, Plasmas and Polymers **6**, 175–188 (2001).
7. R.B. Gadri, J.R. Roth, T.C. Montie, K. Kelly-Wintenberg, Peter P.Y. Tsai, Dennis J. Helfritch, P. Feldman, D.M. Sherman, F. Karakaya, Z. Chen, *Sterilization and plasma processing of room temperature surfaces with a one atmosphere uniform glow discharge plasma (OAUGDP)*, Surface and Coatings Technology **131**, 528–541 (2000).

8. M. Brasoveanu, M.R. Nemtanu, C. Surdu-Bob, G. Karaca, I. Erper, *Effect of glow discharge plasma on germination and fungal load of some cereal seeds*, Romanian Reports in Physics **67**, 617–624 (2015).
9. A. Avram, V. Covlea, A. Matei, M. Bazavan, B. Butoi, B. Bita, E. Barna, A. Jipa, *Impact of RF and DC plasma on wood structure*, Romanian Reports in Physics **67**, 1028–1039 (2015).
10. M. Moisan, J. Barbeau, M.-C. Crevier, J. Pelletier, N. Philip, B. Saoudi, *Plasma sterilization. Methods and mechanisms*, Pure and Applied Chemistry **74**, 349–358 (2002).
11. E. I. Toader, V. Covlea and W. G. Graham, Plasma Sources Science Technol. **15**, 288–293 (2006).
12. A. Tamosiunas, P. Valatkvicius, V. Grigaitiene, V. Valincius, *Operational parameters of thermal water vapor plasma torch and diagnostics of generated plasma jet*, Romanian Reports in Physics **66**, 1125–1136 (2014).
13. J. A. Souza-Correa, C. Oliveira, M. P. Gomes, J. Amorim, J. Phys. D: Appl. Phys. **43**, 395203 (2010).
14. C. Diplasu, Romanian Reports in Physics **66**, 737–745 (2014).
15. O.S. Stoican, Romanian Reports in Physics **66**, 746–753 (2014).
16. X. Deng, A. Yu Nikiforov, P. Vanraes, C. Leys, Romanian Reports in Physics **66**, 1088–1098 (2014).
17. S. Mijović, M. Vučeljić And M. Šćepanović, Romanian Reports in Physics **66**, 481–486 (2014)
18. S. Dobrea, I. Mihaila, V. Tiron, G. Popa, *Optical and mass spectrometry diagnosis of a CO_2 microwave plasma discharge*, Romanian Reports in Physics **66**, 1147–1154 (2014).
19. M Teodorescu, M Bazavan, E R Ionita, G Dinescu, Plasma Sources Sci. Technol. **21,** 055010, (2012).
20. N. Ekem, T. Akan, Y. Akgun, A. Kiremitci, S. Pat, G. Musa, *Sterilization of Staphylococcus aureus by atmospheric pressure pulsed plasma*, Surface and Coatings Technology **201**, 993–997 (2006).
21. M. Laroussi, *Nonthermal decontamination of biological media by atmospheric-pressure plasmas: review, analysis, and prospects*, Plasma Science **30**, 1409–1415 (2002).