PAPER STERILIZATION BY ATMOSPHERIC PRESSURE DBD DISCHARGE

J. VRAJOVÁ[a*], L. CHALUPOVÁ[b], J. ČECH[b], F. KRČMA[a], and P. SŤAHEL[b]

[a] *Institute of Physical and Applied Chemistry, Faculty of Chemistry, Brno University of Technology, Purkyňova 118, 612 00 Brno,* [b] *Department of Physical Electronics, Faculty of Science, Masaryk University, Kotlarska 2, 61137 Brno, Czech Republic*
vrajova@fch. vutbr.cz

Introduction

For many centuries, paper was the main recording medium all over the world. Paper due to the influence of wide range of agents (chemical composition, climatic conditions, biological agents etc.) undergoes different degradation processes that result in alarming conditions of many historical documents. One of the problem that has to be solved is the paper biodeterioration by microbiological agents.

The microbial contamination of the paper materials is a serious problem of many archives and libraries. Among the microorganisms present in archives, the microscopic fungi, commonly known as moulds, play the most important role[1]. The Czech National Library in cooperation with Czech National Archives studied the microbial contamination of old prints originated from 16th – 17th century. The presence of 78 different fungi was prooved[2]. The most visible effect of the paper biodeterioration due to the fungi are the colour stains that originated either from the presence of the fungi itself (fungi mycelium or spores) or as the result of the fungi metabolites. Other important effects are both mechanical corrosion due to the growth of fungi and enzymatic degradation. All of these processes result in the loss of the paper mass and decrease of the paper strength[3].

Generally, the material sterilization can be achieved by chemical and/or physical means, such as heat, chemical solutions, gases and radiation[4]. Majority of conventional sterilization techniques are associated with some level of damage to the material or medium supporting the microorganisms. This does not present any problem, in case where material preservation is not an issue[5]. However, in case where the preservation of the material is the imperative duty, new techniques have to be developed.

In past year many studies were done in the field of plasma sterilization. For an extensive coverage on the sterilization application in low-pressure plasmas, the reader is referred to reference[6]. An overview of sterilization application in atmospheric pressure plasma was given in reference[7] moreover the influence of the UV on sterilization process is decribed. In recent years, the influence of low-pressure plasma on the biodeteriorated paper and on the paper mechanical and chemical properties was studied[8,9].

Basically, the main inactivation factors for cells exposed to plasma are heat, UV radiation and various reactive species[5,6,10]. Each of the single mechanisms mentioned above is germicidal, but they always occur in combinations in the gas plasma and can enforce the sterilizing effect synergistically[10]. The extent of the influence of each factor depends on the plasma operating parameters (applied electrical power, gas mixture, the gas pressure, etc.)[5].

This contribution presents results of the paper sterilization using atmospheric pressure DBD. Moreover the effect of the plasma treatment on the paper morphology and colorimetric properties were studied.

Experimental

The experiments were carried out in a Plexiglass discharge reactor (Fig. 1) with the dimensions 120 × 118 × 120 mm^3. The discharge burned between two plane metal electrodes, both covered with Al_2O_3 ceramics, 0.5 mm thick. Dimensions of metal electrodes were 40 × 40 mm and dimensions of Al_2O_3 ceramics covering the electrodes were 100 × 100 mm. The distance between electrodes was 4 mm in the case of nitrogen and argon plasmas and 10 mm when helium plasma was used. The sample was fixed in the middle of the discharge gap. The scheme of the experimental set-up is shown in the Fig. 2. High voltage with the frequency of 6 kHz was used for discharge generation. The plasma power density was varied from 83 m W cm^{-3} to 1080 m W cm^{-3}. The working gas flow rate was 3 slm in all cases. The discharge parameters were studied by means of the optical emission spectroscopy. The spectra emitted by the discharge were recorded with the Jobin-Yvon TRIAX 550 monochromator equipped with 1200 gr mm^{-1} and 3600 gr mm^{-1} grattings and liquid nitrogen cooled CCD detector. The spectras were recorded in range 200–960 nm. From optical emission spectra, some plasma parameters such as vibrational and rotational temperatures were determined.

Aspergillus niger F8189, the fungi which is commonly found in libraries and archives[1,2], has been chosen as a bioindicator to evaluate the plasma microbial inactivation. The fungi was obtained in Czech collection of microorganisms (Masaryk University Brno – Faculty of Science). The culture of *Aspergillus niger* was cultivated on wort agar (wort + agar powder Himedia RM 026). The spore suspension was prepared by pouring 5 ml sterile water with Tween 80 into the Aspergillus culture and the surface was gently scraped with a wire. The obtained spore suspension was centrifuged three times and the supernatant was discarded. The spore suspen-

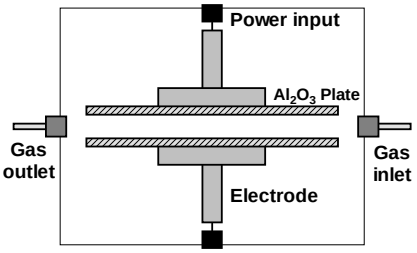

Fig. 1. **Scheme of DBD reactor**

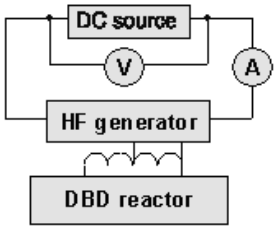

Fig. 2. **Scheme of the experimental set-up**

sion was diluted in sterile water with Tween 80 in order to obtain suspension containing roughly 10^6 spores/ml (Bürker cell was used for spore counting). Afterward the sterilized Whatman paper no. 1 was placed on wort agar in Petri dish and 100 µl of the prepared spore suspension was inoculated on the paper samples. The samples were incubated for seven days at 25 °C. Before the plasma treatment, the paper samples were reached from agar medium and dried for 24 hours at 25 ° C. After the plasma treatment the samples were immersed into 10 ml of sterile water with Tween 80 and placed overnight on the Horizontal Receptable Shaker in order to wash away the spores from the samples. The spore suspension was diluted and dispersed on wort agar plates. After 72 hours of incubation the number of colony forming units (cfu) was counted (Fig. 3). Quantification of the plasma treatment effect was carried out via the survival factor $S = N_{t,\,CFU}/N_{t,0}$ ($N_{t,\,CFU}$ – number of colony forming units/ $N_{t,0}$ – concetration of the spore suspension placed on wort agar).

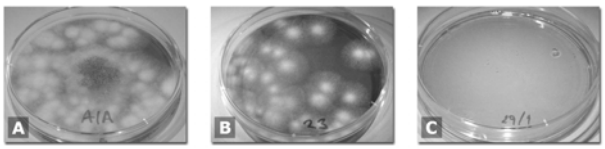

Fig. 3. **Re-cultivated samples after 3 days:** A/ non-treated sample, 70 cfu; B/ sample treated in Argon for 40 s, plasma power density 305.6 m W cm^{-3}, 41 cfu; C/ sample treated in Ar for 240 s, plasma power density 305.6 m W cm^{-3}, 0 cfu

Results

The plasma was studied by optical emission spectroscopy in order to understand the processes, which are present during the plasma sterilization. The OES spectra were taken through the Pyrex glass window, therefore the radiation below 300 nm was absorbed.

The rotational temperature was calculated from the intensities of rotational lines of OH 0–0 system in case of rare gasses discharges and from the structure of rotationally unresolved 0–2 vibrational band of 2nd possitive system of nitrogen in the case of nitrogen discharge[11]. The rotational temperature corresponds to temperature of neutral gas. Rotational temperatures of Ar, He and nitrogen plasmas (given in Tab. I)

Table I
Examples of rotation and vibration temperatures

Gas/power	T_{rot} [K]	T_{vibr} [K]
N_2/ 1083 m W cm^{-3}	400 ± 100	2220 ± 20
He/ 88.9 m W cm^{-3}	290 ± 30	2900 ± 160
He/ 516.7 m W cm^{-3}	310 ± 20	3600 ± 400
Ar/ 83.33 m W cm^{-3}	310 ± 10	1700 ± 200
Ar/ 305,6 m W cm^{-3}	320 ± 10	2300 ± 400

are low enough to ensure the treatment of temperature sensitive samples by means of this plasmas. Vibrational temperature was calculated from the 2nd pos. system of nitrogen ($C^3\Pi_u$ – $B^3\Pi_g$,, $\Delta v = -2$ sequence). The vibrational temperature gives us information about excitation level of plasma. The thermal non-equilibrium of plasma can be seen from the values of the rotational and vibrational temperature given in Tab. I.

The effectiveness of the plasma treatment was reported as the survival factor. The effect of the plasma power input and treatment time using nitrogen plasma has been evaluated from the data shown in Fig. 4. Plasma power density was varied from 262 m W cm^{-3} to 1083 m W cm^{-3}. As it can be seen in the Fig. 4, survival factor decreases with increasing the treatment time. The most significant reduction of the survival factor was obtained within 20 s regardless of the used plasma power density or procces gas (Fig. 5). Further increasing of the treatment time leads only to very slight decrease of the survival factor. This may be due to the „shadowing" effects by spores protecting the underlying layers[12,13]. Moreover, the porosity of the paper has to be considered. The spores may penetrate into the paper material and embed in pits and cavities[14]. Such penetration could preclude the interaction of plasma with the spores, thereby decreasing the efficiency of spore inactivation. Therefore further study has to be done in order to evaluate the contribution of spore concentration and material porosity to the inactivation process. The total removal of the fungi was observed using treatment time of 180 s and plasma power input 1083 m W cm^{-3}. When working with the lower plasma densities longer exposition time is necessary.

Similar results were obtained for all process gases. To compare the inactivation efficiency of nitrogen, argon and helium the plasma power density approximately 300 m W cm^{-3} was used (Fig. 5). The best results were obtained for argon plasma. When operating plasma discharge with plasma densities of 300 m W cm^{-3} the nitrogen plasma seems to be more effective in microorganism inactivation than the helium plasma. For the higher plasma power input, the helium plasma gives much better results, this can be explained by increase of the intensity of O and OH peaks. In order to obtain the same results in nitrogen/ helium as in argon approximately three times (1083 m W cm^{-3})/ two times higher plasma power input (516 m W cm^{-3}) is required.

Possible inactivation processes were provided and commented in the review paper by *Moisan*[6], which included: (*a*) direct destruction of the genetic material of microorganism by UV radiation; (*b*) erosion of microorganisms, atom by atom,

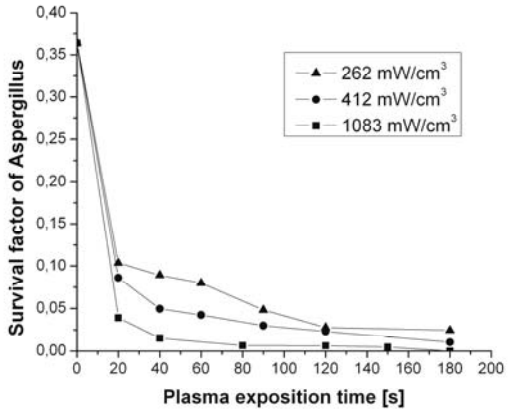

Fig. 4. **Survival factor of Aspergillus niger vs. treatment time, nitrogen was used as a working gas,** plasma power input was varied from 262 m W cm^{-3} – 1083 m W cm^{-3}

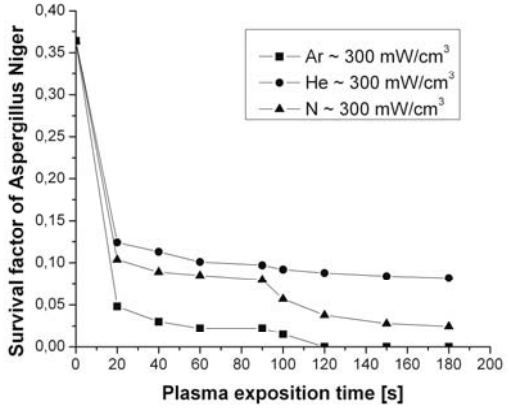

Fig. 5. **Comparison of the inactivation efficiency of nitrogen, argon and helium plasma,** plasma power density approximately 300 m W cm^{-3}

by intrinsic photodesorption; (*c*) erosion of microorganisms, atom by atom, through etching. Taking into account more recent work on low-temperature plasma inactivation at atmospheric pressure, following inactivation mechanisms should be added[7]: (*d*) diffusion of oxygenated species (e.g. OH) through the spore material with ensuing local damage; (*e*) lyses of the bacterium as a results of the rupture of its membrane due to the electrostatic forces exerted on it by accumulation of charged particles coming from the plasma (proved only Gram-negative vegetative spores).

According to *Laroussi*[5], the UV radiation does not play the prominent inactivation role in atmospheric plasmas. Contrary to this statement, *Boudam*[7] proved that spore inactivation, depending on operating conditions, can be achieved either under dominant UV radiation or, on the contrary, under the sole action of the reactive species.

Generally, the most efficient microbial DNA destruction is obtained with the UV radiation in interval 220–270 nm (ref.[15]). Since the significant light intensity losses in our OES occurred in the wavelengths below 300 nm, we are limited to the wavelengths above 300 nm, and thus we are not able to evaluate the influence of the UV radiation on the fungi destruction.

However, the higher inactivation efficiency of argon and helium plasma could result from the presence of impurities in our process gases. The OES spectroscopy prooved the presence of OH radical and atomic oxygen (Fig. 6). These species can contribute to the higher sterilization efficiency as mentioned above. Further the second possitive system of molecular nitrogen was presented in both argon and helium plasma and molecular bands of ionized nitrogen in helium plasma. In nitrogen plasma, only the second positive system of nitrogen was observed in the spectra mainly due to the strong absorption of Pyrex glas below the 300 nm.

Besides the UV radiation and presence of the reactive species, the temperature can play important role during microorganism inactivation. Sterilization by heat is one of the conventional methods used for thermally resistant material sterilization[4]. The contribution of this effect could be low because of low value of rotation temperature (see Tab. I). Except for the nitrogen plasma, the rotation temperature of argon and helium plasma is more or less the same. In the case of nitrogen plasma the temperature can contribute to the plasma sterilization process.

Furthermore the interaction of the DBD with the plain paper material was studied. The paper samples were treated under the same conditions as the samples containing Aspergillus niger spores. Treatment time up to 240 s was used and the total plasma power density was according to the working gas varied in the range 83.33 m W cm^{-3} to 1083 m W cm^{-3}. Afterwards, the colorimetric measurements (colorimeter X-Rite 918) were done to evaluate the changes in paper whiteness and yellowness due to the plasma treatment. The whiteness of the paper decreases with increasing of both the treatment time and/or the plasma power density. Table II. compares the influence of varying plasma power density on the paper colour.

The yellowness of treated paper increased after the plasma treatment, however for argon and helium treated paper

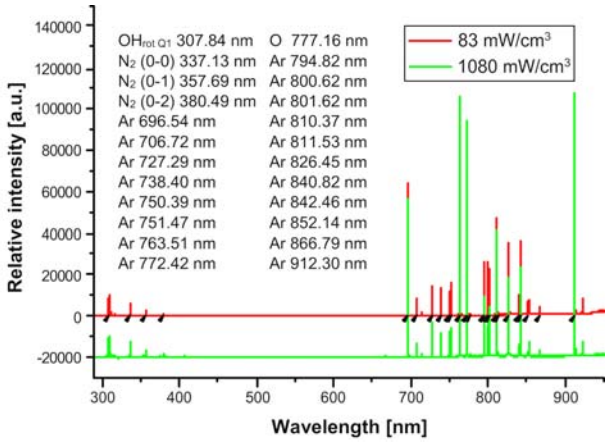

Fig. 6. **OES spectrum of Argon plasma,** plasma power density 83 m W cm^{-3} and 1080 m W cm^{-3}

Table II
Changes of the paper whiteness (Δ WHT) and yellownes (Δ YEL). Paper was treated in Argon for 240 s, minus = decrease, plus = increase

Power [m W cm^{-3}]	Δ WHT	Δ YEL
83.3	–3.87	+1.85
180.6	–4.53	+2.03
305.6	–7.24	+2.34

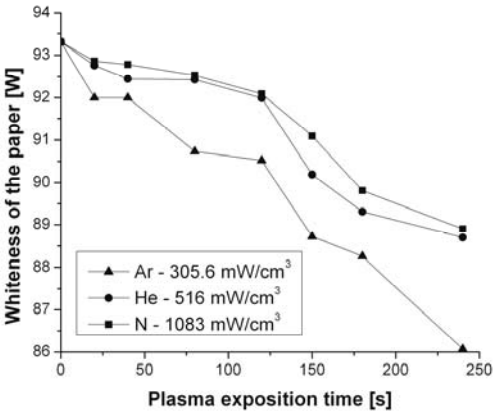

Fig. 7. **The whiteness of the paper after the plasma treatment.** Different plasma power input for each gas is used, in order to compare the samples treated under conditions when the sterilization efficiency was same for all gases

this change was not eye-visible. Fig. 7 compares the influence of different plasma gases on the paper whiteness. The plasma power input, when the same sterilization efficiency was obtained is used for each gas (Ar – 305.6 m W cm^{-3}, He – 516 m W cm^{-3}, N_2 – 1083 m W cm^{-3}). The highest change in the paper whiteness was observed for the argon plasma. According to the literature[16] the paper yellowing and lost of paper strength results from the oxidation of the cellulose fibers. The presence of the atomic oxygen was proved using OES (Fig. 6). The presence of oxygen and nitrogen in OES can give us the assumption on the presence of NO_x species in the discharge, but this statement cannot be proved due to the Pyrex glas spectral cutoff below 300 nm.

Furthermore the structure of the paper was investigated using SEM microscopy. No significant changes were observed after the plasma treatment in the used range of operating conditions.

Conclusion

The DBD discharge generated at atmospheric pressure could be used as the efficient technique for the paper sterilization. The efficiency of the plasma sterilization increases with increasing treatment time and plasma power density. Minimum treatment time over 150 s is required in order to achieve satisfactory sterilization in all used plasmas. The interaction of the plasma discharge with the paper substrate causes the slight decrease of the paper whiteness and yellowness of the paper samples.

Further studies will be done in order to study the sterilization efficiency of DBD on wide spectrum of microorganisms, moreover the influence of the plasma treatment on the chemical and mechanical properties of the paper materials will be studied.

This work was supported by the Czech Science Foundation contracts No. 202/03/H162, by Grant Agency of Academy of Science of Czech Republic contract No. KAN101630651 and by the Research Intent from Ministry of Education, Youth and Sports of Czech Republic No. MSM0021622411.

REFERENCES

1. Skorkovsky B., in: *Microorganisms as the agents of archival documents degradation*, Chap. 1, p. 108. TEPS, Prague, 1981.
2. Odvarkova J., Bacilkova B., in: *Proceedings of 7th Symposium on Historical and Scarce Books of Bohemian, Moravian and Silesian Book Stock, Olomouc, 1998*.
3. Bacilkova B.: Bulletin STOP *6*, 2 (2004).
4. Silhankova L., in: *Microbiology for the Food Industry and Biotechnology*. Victoria Publishing, a.s., Prague 1995.
5. Laroussi M., Leipold F.: Int. J. Mass Spectrom. *233*, 81 (2004)
6. Moisan M., Barbeau J., Moreau S., Pelletier J., Tabrizian M., Yahia L. H.: Int. J. Pharmaceutics *226*, 1 (2001)
7. Boudam M. K., Moisan M., Saoudi B., Popovici C., Gherardi N., Massines F.: J. Phys. D: Appl. Phys. *39*, 3494 (2006)
8. Laguardi L., Vassallo E., Cappitelli F., Mesto E., Cremona A, Sorlini C., Bonizzoni G.: Appl. Surf. Sci. *252*, 1159 (2005).
9. Vohrer U., Trick I., Bernhardt J., Oehr C., Brunner H.: Surf. Coatings Technol. *142–144*, 1069 (2001).
10. Muranyi P., Wunderlich J., Heise M.: J. Appl. Microbiology *103*, 1535 (2007)
11. Janca J., Skricka L., Brablec A.: Plasma Chem. Plasma Process. *13*, 3 (1993).
12. Heise M., Neff W., Franken O., Muranyi P., Wunderlich J.: Plasmas and Polymers *9*, 23 (2004)
13. Muranyi P., Wunderlich J., Heise M.: J. Appl. Microbiology *103*, 5 (2007).
14. Rogers J. V., Sabourin C. L. K., Choi Y. W., Richter W. R., Rudnicki D. C., Riggs K. B., Taylor M. L., Chang J.: J. Appl. Microbiology *99*, 4 (2005).
15. Moisan M., Bareau J., Crevier M. C., Pelletier J., Phillip N., Saudoni B.: Pure Appl. Chem. *74*, 349 (2002).
16. Durovic M., in: *Restoration and Conservation of the Archival Documents and Books*, Chap. 2.1.5., p. 517. Paseka, Prague 2002.

J. Vrajová[a*], L. Chalupová[b], J. Čech[b], F. Krčma[a], and P. Sťahel[b] *([a] Institute of Physical and Applied Chemistry, Faculty of Chemistry, Brno University of Technology, Brno, [b] Department of Physical Electronics, Faculty of Science, Masaryk University, Brno, Czech Republic):* **Paper Sterilization by Atmospheric Pressure DBD Discharge**

In this paper, the removal of the microbial contamination from paper material using the plasma treatment at atmospheric pressure is investigated. The *Aspergillus niger* has been chosen as a bio-indicator enabling to evaluate the effect of plasma assisted microbial inactivation. Dielectric barrier discharge (DBD) operated at atmospheric pressure was used for the paper sterilization. The working gas (nitrogen, argon and helium), plasma exposition time and the plasma power density were varied in order to see the effect of the plasma treatment on the fungi removal. After the treatment, the microbial abatement was evaluated by the standard plate count method. This proved a positive effect of the DBD plasma treatment on fungi removal. Morphological and colorimetric changes of paper substrate after plasma treatment were also investigated.