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STUDY OF STERILIZATION EFFECT OF DIELECTRIC BARRIER DISCHARGE ON EUCARYOTIC MICROORGANISMS

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Abstract. The work was focused on studying of the effect of the dielectric barrier discharge (DBD) operating at atmospheric pressure on bioindicator *Aspergillus niger*. Plasma was generated in nitrogen and argon. Paper and PET-foil were used as the carrying medium. The influence of various working conditions on the efficiency of plasma sterilization was studied. Some partial results are presented.

1. INTRODUCTION

Nowadays the wide spectrum of decontamination methods are used for the inactivation of microorganisms on various materials and subjects (Silhankova et al. 1995). The serious disadvantage of the conventional decontaminations methods is stressing of the exposed material by heat or chemicals (Muranyi et al. 2007, Heise et al. 2004, Laroussi et al. 2004). Plasma sterilization is the alternative method, which is more friendly and more effective on the wide spectrum of procaryotic and eucaryotic microorganisms (Muranyi et al. 2007). Basically, the main inactivation factors for cells exposed to plasma are heat, UV radiation and various reactive species (Heise et al. 2004, Laroussi et al. 2004). Many scientists test the possibilities of plasma sterilization in a wide spectrum of applications. Mainly it is food industry (sterilization of spices, almonds or packaging materials) (Heise et al. 2004, Schneider et al. 2005, Deng et al. 2007) and health science (the effect at healing of wounds, sterilization of instrumentation) (Ehlbeck et al. 2008, Weltmann et al. 2009).

Plasma sterilization enables measuring at different conditions. Various type of plasma generation can be employed, e.g. in recent years a number of papers has been published on plasma sterilization using DBD (Muranyi et al. 2007, Heise et al. 2004, Laroussi et al. 2004) or microwave plasma (Schneider et al. 2005, Ehlbeck et al. 2008, Lee et al. 2005). It is possible to use low pressure (Schneider et al. 2005, Lee et al. 2005) or atmospheric pressure (Muranyi et al. 2007, Heise et al.

2004, Ehlbeck et al. 2008), the plasma sterilization can be operate in different atmospheres of gases (nitrogen, argon, helium, air,...) (Heise et al. 2004, Laroussi et al. 2004, Lee et al. 2005), it is also possible to choose the proper carrying medium (paper, polymers or glass) (Muranyi et al. 2007, Heise et al. 2004, Weltmann et al. 2009, Lee et al. 2005).

This contribution presents results of the plasma sterilization using atmospheric pressure DBD. The goal of our work was to specify the best conditions for the inactivation of model organism, the fungi *Aspergillus niger*. Namely it was the influence of plasma exposition time, plasma power density, the type of operating gas and type of the medium supporting the microorganism.

2. EXPERIMENTAL

Aspergillus niger F8189 has been chosen as a bio-indicator 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 10 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 suspension was diluted in sterile water with Tween 80 in order to obtain suspension containing roughly 10^7 spores/ml. Afterward the sterilized Whatman paper no. 1 (or the polymer foil) was placed in Petri dish and 100 µl of the prepared spore suspension was inoculated on the samples. The samples were dried for 24 hours at 25 °C. Afterwards the dried samples were treated in DBD.

The microbial abatement was evaluated before and after the plasma treatment by using the standard plate count method. The treated 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. From the observed results the survivor curves, the plots of the logarithm of the number of colony forming units per unit volume versus plasma exposition time, were constructed.

The experimental method is based on the principle of volume DBD. The experiments were carried out in a Plexiglass discharge reactor with the dimensions 120 × 118 × 120 mm. The discharge burned between two plane metal electrodes, both covered with mica, 0,5 mm thick. Dimensions of metal electrodes were 90 x 70 mm. The distance between electrodes was 3 mm. The sample was fixed in the middle of the discharge gap. High voltage with the frequency of 10 kHz was used for discharge generation. The plasma power density was varied from 1 153,44 $mWcm^{-3}$ to 2 562,96 $mWcm^{-3}$. 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 and 3600 gr/mm

grattings and liquid nitrogen cooled CCD detector. The spectras were recorded in range 200 – 960 nm.

3. RESULTS AND DISCUSSION

The goal of our work was to specify the decontamination conditions for the bioindicator *Aspergillus niger*. The standard plate count method was used to evaluate the inactivation effect of various operating conditions operating conditions (working gas, exposition time, plasma power density) and medium supporting the microorganism

The efficiency of various gases is not the same, because of the different composition. Plasma releases the free radicals, which have the negative effect for the microbial cells. When we compare two gases, nitrogen and argon, we find out, that argon has the higher inactivation effect than nitrogen, even at the lower plasma power density (Fig. 1). It is possible to reduce the microbial contamination about 4 – 5 orders within 120 seconds (plasma power density 1 164,02 mW·cm^{-3}). When using higher plasma power density better results (5-6 orders of magnitute) were observed. In order to achieve the same sterilization effect in nitrogen plasma as in argon the higher plasma power density (2 044,44 mW·cm^{-3}) has to be used.

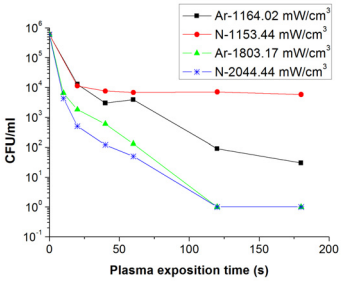

Figure 1: The comparison of the sterilization effect of various gases (nitrogen and argon) and plasma power input.

OES was performed in order to characterise the DBD and to evaluate the presence of plasma species responsible for antimicrobial effects, such as free radicals and UV-emitting particles. The spectrum of argon has the strong emission about 308 nm, because of existence of OH-system. It is also observed the 2nd pos. system of nitrogen (337 nm, 357 nm and 380 nm) and CN-system in range of 385 – 389 nm. Furthermore argon lines (above 690 nm) and the atomic oxygen lines (777 nm) were detected. The spectrum of nitrogen shows similar as argon the OH-system in the area 306 – 310 nm and the 2nd pos. nitrogen system. It is possible to observe the emission of NOγ molecular band (220 – 290 nm). Generally, the most efficient microbial DNA destruction is obtained with the UV radiation in interval 220 – 270 nm (Silhankova et al. 1995). The emission in these range was detected only in nitrogen spectra but not in argon spectra. Nevertheless the better sterilization effect was observed in argon plasma, this can be result of the presence of OH radical and atomic oxygen, these species can contribute to the higher sterilization efficiency.

Every carrying medium has the specific properties, and thus different effect of the plasma treatment can be observed. In order to evaluate the influence of carrying medium on inactivation process paper and polymer foil was used as a carrying medium. In case of the paper the porosity has to be considered. The fungi spores may penetrate into the paper material and embed in pits and cavities, such penetration could preclude the interaction of plasma with the spores, thereby decreasing the efficiency of spore inactivation. Moreover it can make impossible to wash away the spores from the paper. On the other hand the PET foil has the smooth surface, and thus provides no protection for microbial cells. For this especial property is PET the mostly used packing material, especially for food industry (Heise et al. 2004). The results are summarised in Tab. 1. It can be seen that the contamination on the polymer foil is minimal about 1 - 2 orders higher and the inactivation runs slowly. In opposite, paper decontamination runs fast. But due to the impossibility to wash out all spores is this state apparent.

Table 1. The comparison of the efficiency of plasma sterilization with use of various carrying mediums

time [s]	0	10	40	60	120
paper [CFU/ml]	$8{\cdot}10^4$	$5{\cdot}10^3$	$6{\cdot}10^2$	0	0
PET [lCFU/ml]	$4{\cdot}10^5$	$2{\cdot}10^5$	$2{\cdot}10^4$	$2{\cdot}10^4$	0

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