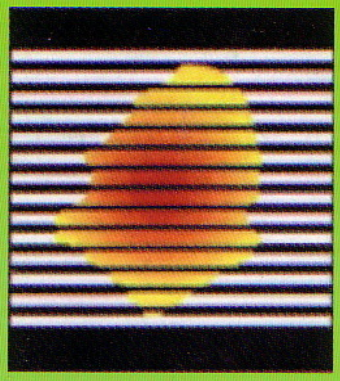

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BIOLOGICAL EFFECTS OF DC DISCHARGES IN ATMOSPHERIC AIR WITH WATER

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ABSTRACT. We tested biological effects of three types of DC electrical discharges in atmospheric air with one electrode submerged in water. The studied discharges were streamer corona, transient spark and glow discharge, operating in non-homogeneous electric field in the point-to-plane configuration, in both polarities of DC high voltage applied. They generate non-thermal plasmas with various gas temperatures and levels of thermal non-equilibrium. The discharge properties were investigated by means of electrical measurements and optical emission spectroscopy. Their biological effects were tested on selected Gram-negative and Gram-positive bacteria cultures (*S. typhimurium* and *B. subtilis*). The comparison of the effects of the three discharges indicates the major role of radicals and active species among other inactivation mechanisms.

1. INTRODUCTION

Non-thermal plasmas at atmospheric pressure are nowadays widely investigated for various chemical (flue gas and water cleaning) and bio-decontamination (sterilization) applications. [1, 2] Despite most related works use pulsed coronas and dielectric barrier discharges, we investigate three types of DC atmospheric discharges and test their biological effects on selected bacteria. In sterilization, it is very important to assess the importance of various effects involved. [2] We attempt to do so by employing emission spectroscopy of the investigated discharges.

2. INVESTIGATED DC DISCHARGES

2.1. Experimental-setup

The experimental setup for investigations of the DC discharges is shown in Figure 1. A homemade DC high voltage power supply (max. 25 kV/30 mA) sustains the discharges, enables mean voltage and current monitoring, and easy modification of the ballast resistance. The discharges were operated in the discharge chamber that allows their electrical, optical and spectroscopic diagnostics, working in various gases, with or without the plate electrode submerged in water, and changing the interelectrode distance. The stressed high voltage electrode was a hollow needle, opposite to the plate counter-electrode.

The discharge voltage was measured by the high voltage probe Tektronix P6015A (1000x). In the transient spark, the internal capacity of this high voltage probe was filtered by a small separating resistor *r* in order to minimize the capacity discharging in the spark pulse. A careful correction of the measured voltage on this *r* was then done. The interelectrode distance was varied between 2-10 mm. The discharge current was measured by: 1) mean DC current with

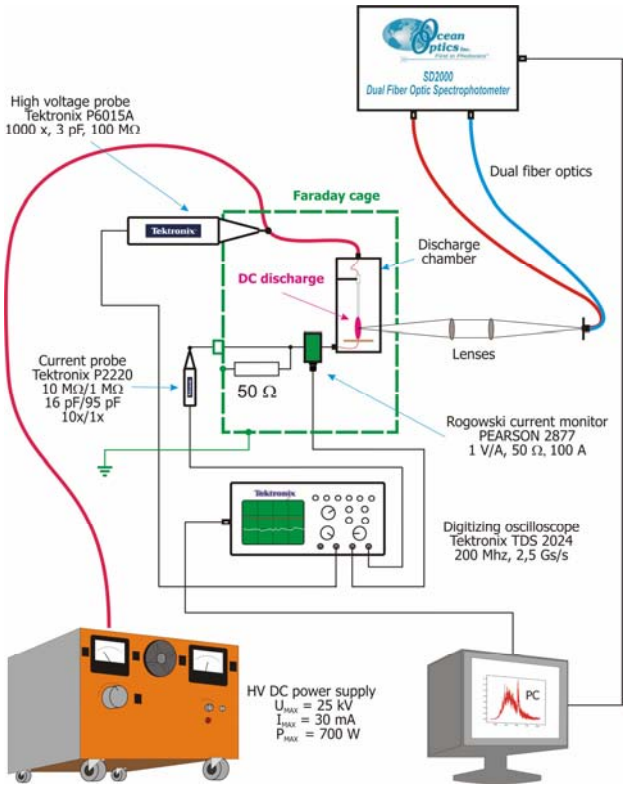

Figure 1. Experimental set-up for DC discharge experiments

milliamp meter; 2) time-dependent current waveform by the current probe on a 50 Ω resistor; and 3) by a Rogowski current monitor PEARSON 2877 (1 V/A). The current and voltage signals were processed by a digitizing oscilloscope Tektronix TDS 2024 (4 channels, 200 MHz, 1 GS/s) communicating with a PC. The entire chamber was placed in a Faraday cage to reduce induced noise signals and correctly measure voltage and current waveforms.

The light emission from the discharges was lead through the optical system consisting of a He-Ne laser for the alignment, an iris and two lenses, and focused on the entrance of the Y-type fiber optic leading into two-channel spectrometer Ocean Optics SD2000 (Master 200-500 nm, resolution 0.8; and Slave 500-1100 nm, resolution 1.7 nm) that was used for the optical emission diagnostics. The discharges were also photo-documented with a digital camera.

2.2. Electrical discharge characteristics

Three types of DC discharges in both polarities operating in atmospheric pressure air with or without water present in the discharge chamber were investigated: classical ***streamer corona (SC),*** and relatively novel ***transient spark (TS)*** and ***glow discharge (GD)***. These discharges generate nonequilibrium plasmas inducing various chemical end biological effects that are in the interest of chemical and bio-decontamination. Each discharge type generates plasma with specific properties, so each one was studied separately, both in the positive and the negative polarity. Testing discharges with an electrode immersed in water is important in order to adapt the conditions to the discharge action on biological media in water solution. The discharges have already been extensively studied previously. [3, 4] They are photo-documented in Figure 2.

Due to limited space we only show here the typical SC and TS pulses (Figure 3) of very short (~100 ns) duration. The discharge voltage remains fairly constant during the small streamer current pulses, while with TS it drops to zero during the high current pulses (~1A). The typical repetitive frequency of the streamer and spark pulses is 0.5-5 kHz, and 10-30 kHz, respectively. Thanks to very short pulse duration in TS (given by a small internal capacity of the discharge chamber), the plasma cannot reach LTE conditions. [3, 5]. On the other hand, the periodic streamer-to-spark transition provides nonequilibrium conditions with fast electrons that result in efficient chemical [3, 5] and biological cleaning effects. Pulseless high pressure DC glow discharges between two metal (or metal-liquid) electrodes and their chemical effects have been already described [3-6].

streamer corona transient spark glow discharge

Figure 2. Photographs of the discharges at various exposures, gap distance 4 mm.

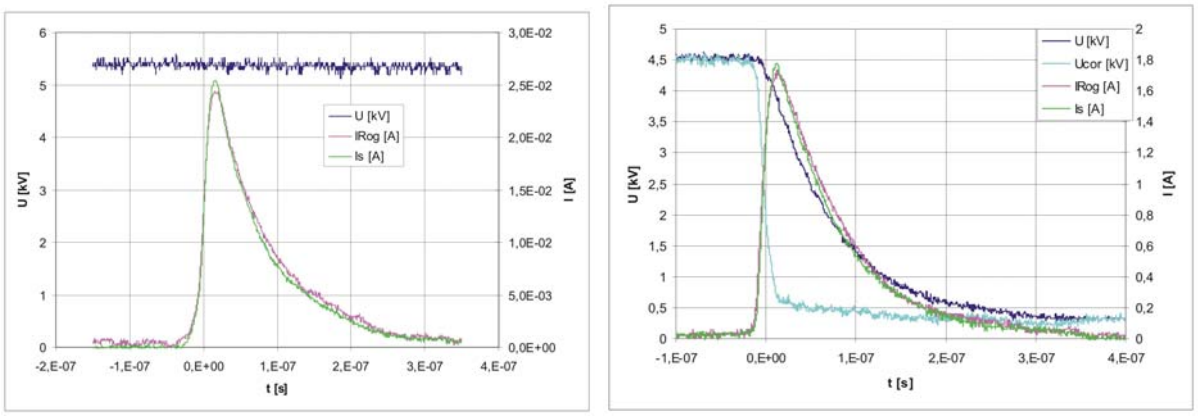

Figure 3. Streamer corona (left) and transient spark (right) – voltage and current pulses. U_{cor} is the voltage corrected for the value of the separating resistor r, I_{Rog} measured by the Rogowski current monitor, I_s measured by the current probe on a 50 Ω resistor.

2.3. Emission spectroscopy of DC discharges

Emission spectroscopy in UV-VIS region is a powerful technique of plasma diagnostics, because it gives valuable information on excited atomic and molecular states, enables to determine the rotational and vibrational temperatures and thus the level of nonequilibrium in the plasma, and, in addition, gives insight in an ongoing plasma chemistry [7].

Typical acquired spectra of the three discharges are shown in Figure 4. All discharges emit N_2 (2^{nd} and 1^{st} positive system), which indicates the excitation of N_2 molecules into $C^3\Pi_u$ state by the impact of electrons with relatively high energy (11 eV), and into $B^3\Pi_g$ state by electron impact or by C-B de-excitation. All discharges provide OH emission, thus indicating the presence of excited OH radicals formed from water molecules by dissociation.

GD emits the strongest OH and also emits NO γ system. NO is formed by the reaction N+O at high temperatures (~2000 K). Therefore, N and O radicals must be formed by the dissociation of air components. In addition, there is a strong water evaporation and consequent H_2O dissociation, thus leading to the large OH population. TS, besides N_2, emit N_2^+, which signifies high electron energy (19 eV is needed to excite N_2^+ into $B^2\Sigma_u^+$ state). High energy electrons leave

their fingerprints also in the VIS spectra as atomic lines of O, N and H radicals. These were not observed with other discharges. There is still a strong water dissociation and OH radical formation. SC only emits N_2 (2^{nd} and 1^{st} positive system) and some weak OH.

The measured spectra were used to determine the vibrational T_v and rotational T_r temperatures after fitting the experimental spectra with the simulated ones. We use Lifbase [8] (for OH) and Specair [9] (for N_2 a OH) simulation programs. Owing to fast collisional relaxation at atmospheric pressure, the gas temperature $T_g \approx T_r$. $T_v >> T_r$ indicates the nonequilibrium in the plasma. The preliminary results of temperature measurements are the following:

Streamer corona	$T_g \approx T_r = 500 \pm 200$ K,	$T_v = 6000 \pm 1000$ K
Transient spark	$T_g \approx T_r = 1000 \pm 500$ K,	$T_v = 8000 \pm 1000$ K
Glow discharge	$T_g \approx T_r = 2000 \pm 500$ K,	$T_v = 4000 \pm 1000$ K

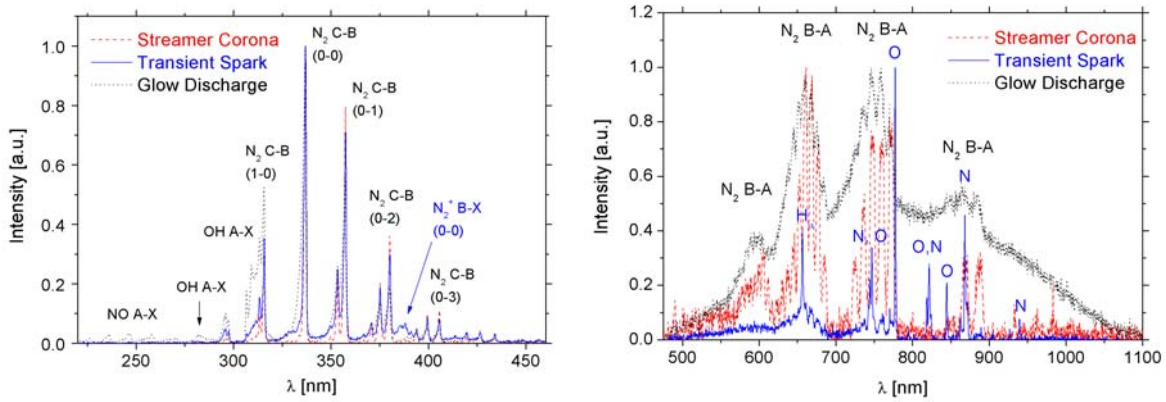

Figure 4. Emission spectra of DC discharges in UV and VIS.

3. BIOLOGICAL EFFECTS OF DC DISCHARGES

The biological effects of investigated DC discharges have been preliminarily tested on selected bacteria cultures:

1) *Salmonella typhimurium*, genetically modified strain (stem) TA 98, Gram-negative bacteria,

2) *Bacillus subtilis*, Gram-positive bacteria.

S. typhi is a pathogen causing typhus diseases, and so its sterilization is important from the viewpoint of drinking water decontamination. *B. subtilis* is not dangerous but belongs to the same group as extremely hazardous *B. anthracis* (Anthrax precursor), which nowadays represents one of the highest bio-terrorism risks. Besides the sterilizing effect of the non-thermal plasmas generated by the discharges, we partially investigated the roles of the involved bio-inactivation mechanisms, such as the thermal effect, the effect of radicals, charged particles, the electric field, and of the UV radiation.

3.1. Bacteria handling cultivation procedure

We used a standard microbiology procedure of bacteria handling and cultivation. The bacterial culture kept in the refrigerator was inoculated in the liquid nourishing medium. After pH adjustment it was placed in the overnight shaker at 37 °C where bacteria multiplied their

population. A diluted bacterial solution – inoculum – was then prepared with exactly set concentrations, typically measured in colony forming units (CFU) per ml, in the physiological medium. McFahrland turbidity scale standards were used to set the initial concentration. An exact volume (typically 10 µl) was then inoculated onto 4 Petri dishes with sterilized agar to make reference samples. The other part of inoculum (5 ml) was put in the discharge dish and treated by the specific discharge for a certain time (15, 30 or 60 s). After the discharge action, an exact volume of the treated inoculum was applied on other 4 Petri dishes with agar. All reference and treated samples on Petri dishes were incubated at 37 °C during 24 h to cultivate colonies (CFUs). Each living bacteria multiplies and gives rise to a colony observable by a naked eye. After the incubation, colonies on all Petri dishes were counted and statistically evaluated.

We developed a special discharge chamber for biological tests consisting of a 4-cm diameter Petri dish with a round copper plate counter-electrode on the bottom. The dish is placed in a glass vessel with a cover equipped with the needle electrode. The treated bacterial inoculum of the exact volume (typically 5 ml) is applied in the dish, onto the plate electrode. The discharge burns from the needle directly into the bacterial solution.

3.2. Results of bio-decontamination effects

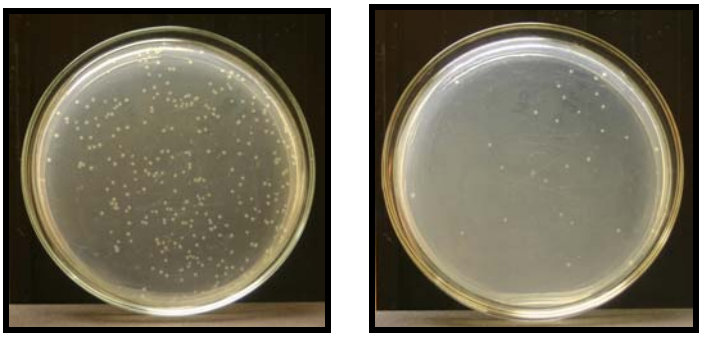

Figure 5. Cultivated bacteria on Petri dishes. Reference (left) and after-treatment (right) samples.

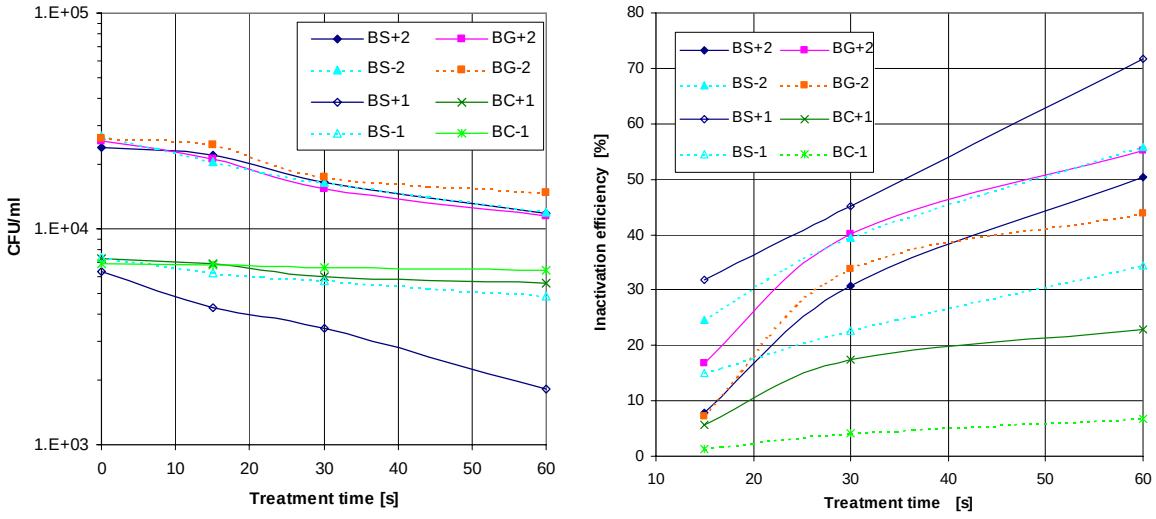

Figure 6. *S. typhi* survival curves in semi-logarithmic scale (left) and inactivation efficiency vs. treatment time (right). BS: transient spark, BG: glow discharge, BC: streamer corona, +: positive, -: negative polarity.

Several sets of bio-experiments have been performed. One set typically contained the treatment of the same inoculum by one discharge type in both polarities for treatment times of 15, 30, and 60 s, each combination per four samples, and 4 reference samples. The example of reference and treated cultivated samples is shown in photographs in Figure 5.

The survival curves of *S. typhi* are shown in Figure 6 in semi-logarithmic scale. The graph shows 4 experiment sets, two starting at lower (7000 CFU/ml – denoted 1) and two at higher (26,000 CFU/ml – denoted 2) initial bacterial concentrations. As expected, the number of CFUs decreased with the treatment time with all discharges. However, faster or slower inactivation can be observed with different discharges. In order to better compare the discharges we express the relative concentration decrease, i.e. the inactivation efficiency (Figure 6 right). The highest efficiencies were obtained in the positive TS, the lowest in the coronas; the GD gives fairly high efficiencies as well. All tested discharges were more efficient in the positive polarity.

Despite we performed several tests of inactivation of the Gram-positive spore-forming *B. subtilis* bacteria, so far we were not able to reasonably evaluate the survival curves and the efficiency. It is due to the problem with CFU counting after incubation of the tested and the reference samples. Although the initial concentrations were low, the incubated bacteria on the Petri dishes did not form nicely distinguished CFUs but continuous stains. However, a large decrease of their concentration was visually apparent which confirms that the method is applicable even for Gram-positive bacteria that are in general harder to inactivate.

3.3. Inactivation mechanisms

Bacteria inactivation (sterilization) mechanisms differ according to different authors. Most of them find that at atmospheric pressure, radicals and other active particles produced in the discharges play the dominant role. [2]

Our results obtained on inactivation of Gram-negative *S. typhi* demonstrate the highest efficiency in the positive TS. Spectroscopic characteristics of the TS described earlier showed that electrons with high energies are present in this discharge. These electrons initiate dissociations, ionizations and excitations of various species. The plasma is highly nonequilibrium, since $T_v >> T_r$. The atomic O, N and H radicals, and the N_2^+ ions have only been detected in this discharge, and there were a lot of OH radicals. O radicals may react with air O_2 and form ozone O_3. These results indicate that the role of radicals and other active species is very important. Energetic electrons themselves, directly interacting with bacteria may be important as well. High inactivation efficiencies were also reached in GD but at higher energy costs, due to the gas heating. The advantage of GD is a large number of OH radicals forming by dissociation of the vaporized water. It is hard to distinguish the temperature effect at this stage. SC was the least efficient discharge. This is partly because it is the least energetic and partly because the active region is in the proximity of the needle tip only, while most of the space is occupied by the drift region. Corona has the strongest electric field compared to other investigated discharges but this seems to have a minor effect.

At the present we are not able to distinguish the effect of UV radiation from the discharges. The most intense radiation comes from TS, mainly in UV A and VIS. On the other hand, GD emits more UV B germicide radiation due to NO γ system. This effect will be further investigated. We also plan experiments comparing direct and remote exposure of the micro-organisms in order to better separate the effects of active species from the effects of heat, UV, electric field and charges particles.

4. CONCLUSIONS

Three types of DC electrical discharges in atmospheric air with one electrode submerged in water (streamer corona, transient spark and glow discharge) were investigated by electrical measurements and optical emission spectroscopy. They generate non-thermal plasmas with various gas temperatures and properties. We performed the fist tests of their biological effects on selected Gram-negative and Gram-positive bacteria cultures (*S. typhimurium* and *B. subtilis*). The obtained bio-decontamination gives satisfactory results, although process optimization is necessary to reach higher inactivation efficiencies. In the synthesis with the spectroscopic discharge investigations we were able to determine some important mechanisms in the bio-decontamination process, especially the major role of radicals and active species. The preliminary results obtained are important from the standpoint of wastewater disinfection or bio-decontamination.

ACKNOWLEDGEMENTS

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