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VARIOUS DC DISCHARGES FOR STERILIZATION AT ATMOSPHERIC PRESSURE

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Abstract: Three types of DC electrical discharges in atmospheric air (streamer corona, transient spark and glow discharge) were tested for sterilization of bacteria in water solution, and spores on surfaces. Static vs. flowing regime treatment of contaminated water were compared, in the latter the flowing water either covered the grounded electrode or passed through the high voltage needle electrode. The bacteria were killed most efficiently in the flowing regime by transient spark. Streamer corona was efficient when the treated medium flew through the active corona region. The spores on plastic foil and paper surfaces were successfully sterilized by negative corona. The bacteria and spores were handled and their population evaluated by standard microbiology cultivation procedures. The emission spectroscopy and TBARS (thiobarbituric acid reactive substances) method (spectrometric detection of the products of lipid peroxidation) indicated a major role of radicals and reactive oxidative species in the bio-decontamination mechanisms.

1. INTRODUCTION

Atmospheric pressure non-thermal plasmas are nowadays widely investigated for various bio-medical applications, especially sterilization [1-3]. We investigate three types of DC atmospheric discharges and test their bio-decontamination (sterilization) effects on selected bacteria in water solutions and spores on surfaces. In bio-decontamination, it is very important to assess the role of various mechanisms involved [1]. We identify the dominant mechanisms by comparing the electrical characteristics of the investigated discharges, their emission spectra, sterilization effects in various discharge and flow regimes, and TBARS detection of oxidative stress of bacterial cells.

2. EXPERIMENT

2.1. Experimental setups

The experimental setup for investigations of the DC discharges was shown elsewhere [4-6]. The sterilization effects of these discharges were tested in both static and flowing regimes. In the static regime, the discharges were operated in point-to-plane geometry, with high voltage needle electrode and the plane electrode submerged in a certain volume (typically 5 ml) of contaminated water. Fig. 1 shows the setup for the continual flowing water treatment. Five parallel discharges were operated in a transparent discharge tube. The stressed high voltage electrodes were hollow needles, opposite to the copper plate electrode submersed in a water stream, with the typical needle-water distance of 4 mm. The water flow rates, and thus the residence times in the discharge tube, were varied.
Another flowing regime arrangement was developed to increase efficiency of corona discharge. It enables flowing the treated medium directly through the high voltage needle electrode, and so through the corona active region in its proximity. The effect of electrostatic spraying occurred due to the lowering of the water surface tension when the high voltage was applied on the needle electrode. Fig. 2 shows the set-up and demonstrates the water spraying effect through the corona active zone.

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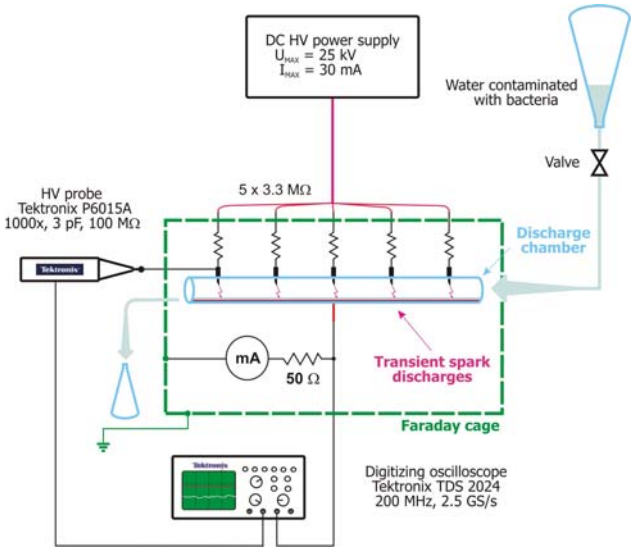


FIGURE 1. Experimental set-up and a discharge tube with 5 parallel discharges for flowing regime water treatment.

FIGURE 2. Flowing the contaminated water through the high voltage needle electrode and electrospraying effect in the corona discharge.

We used point-to-plane electrode geometry for the sterilization of spores on dielectric surfaces (plastic foil, paper). The needle electrode was placed about 4-5 mm above the spore stain on the surface and negative high voltage was applied for 5 or 10 minutes. The treated surfaces were placed on the grounded plane electrode.
The discharge voltage on needle electrodes was measured by a high voltage probe Tektronix P6015A. The discharge current was measured on a 50 Ω or 1 Ω resistor; and by a Rogowski current monitor PEARSON 2877. The current and voltage signals were processed by a digitizing oscilloscope Tektronix TDS 2024 (200 MHz). The light emission from the discharges was detected by a two-channel spectrometer Ocean Optics SD2000 (200-1100 nm, resolution 0.6-1.2 nm).

2.2. Applied DC Discharges

Three types of DC discharges of both polarities operating in atmospheric air with water were investigated: well-known streamer corona, and relatively novel transient spark (TS) and glow discharge (GD). These discharges generate non-equilibrium plasmas inducing various chemical and biological effects that play role in bio-decontamination. They were described in our previous work [6]. We only mention here the negative corona that was employed for sterilization of spores on surfaces. The discharge appearance is shown in Fig. 3. The negative corona above plastic foil demonstrated typical Trichel pulses with frequencies up to 5 MHz and amplitudes of 0.25 mA. The frequency of pulses increased with the applied voltage, up to a certain limit when the pulsed mode transited into a pulse-less mode of constant low current (0.14 mA). When paper was placed on the grounded electrode, the discharge partially penetrated into its porous structure with surface microdischarges forming, similar to back corona that forms on highly resistive porous layers covering the low voltage electrode. The corresponding discharge pulses were higher (16 mA) but with lower frequency (1 kHz).

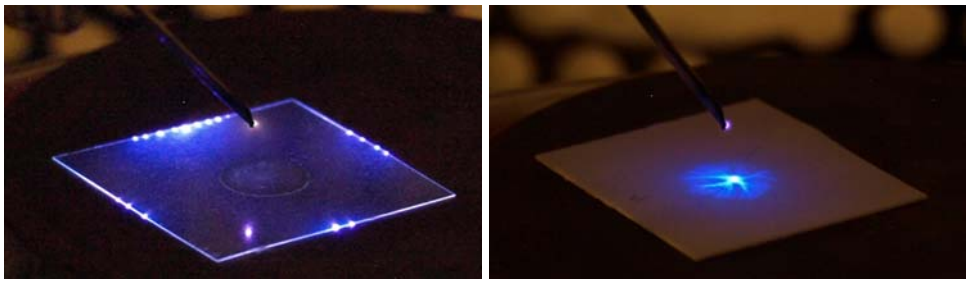


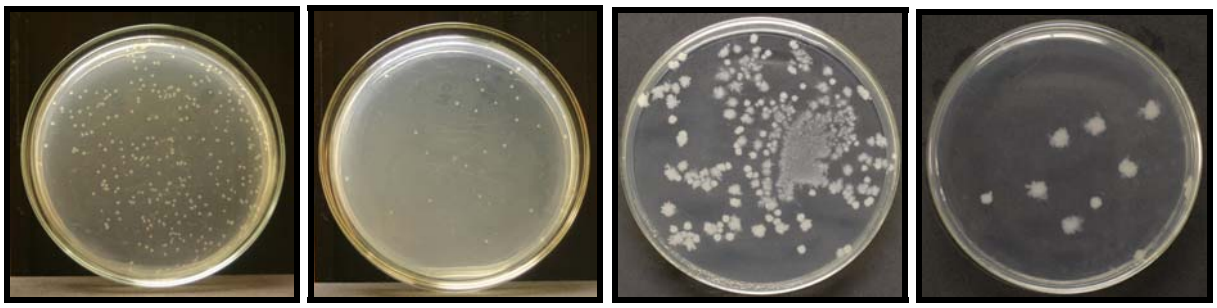
FIGURE 3. Negative corona above plastic foil (left) and paper (right) surfaces, gap distance 4 mm.

2.3. Treated bacteria

Biological effects of investigated DC discharges were tested on selected bacteria in water and spores on the surfaces (plastic foil, paper). The bacterial survival rate (inactivation) was examined by standard cultivation methods of a thermostatic growth on agar in Petri dishes, and was statistically evaluated. The bacteria cultivated on Petri dishes are illustrated in Fig. 4. The following bacteria and spores were treated:

1) *Salmonella typhimurium*, Gram-negative bacteria, (genetically modified strain TA 98): pathogen causing salmonelosis diseases; its inactivation is important for drinking water decontamination.

2) *Bacillus cereus*, Gram-positive bacteria (and spores): belongs to the same group as extremely hazardous *B. anthracis* (Anthrax precursor) that nowadays represents a high bio-terrorism risk.



a Salmonella typhimurium b c Bacillus cereus d

FIGURE 4. Cultivated bacteria on Petri dishes. Reference *(a,c)* and after-treatment *(b,d)* samples.

3. RESULTS AND DISCUSSION

3.1. Static regime treatment

The survival curves of *S. typhimurium* in water or physiologic solution treated in the static regime were shown previously [4]. The bacterial population decreased with the treatment time in all discharges. We express the relative population decrease, i.e. the *inactivation efficiency*. The highest efficiencies (72%) were obtained in the positive TS, the lowest in the coronas; GD gave fairly high efficiencies as well.

Conventional sterilization methods are often evaluated by *D-value* (decimal value) that represents the time of the microbial population reduction by 90 % (1 order of magnitude). The achieved D-values varied from 1.8 min (TS) to 32 min (corona). We also tested the inactivation of the *B. cereus* in the static regime with efficiency 98.7% obtained after 60 s treatment in TS.

3.2. Identification of mechanisms: Optical Emission Spectroscopy (OES)

OES in UV-VIS region is a powerful technique of plasma diagnostics - it gives valuable information on excited atomic and molecular states, enables to determine the rotational and vibrational temperatures, and gives insight in ongoing plasma chemistry [5]. We employed OES, along with the comparison of the induced bio-effects to identify the dominant mechanisms involved in sterilization.

The discharges emit N_2 (2^{nd} and 1^{st} positive system) and OH, thus indicating the presence of excited OH radicals formed by dissociation of water molecules. GD emits the strongest OH and also NO γ system. TS emits in addition N_2^+ and atomic lines of O, N, and H radicals that signify high electron energy. The typical UV and VIS-NIR spectra and evaluated rotational and vibrational temperatures are detailed in [5]. SC and TS generate cold, nonequilibrium plasmas (300-550 K), GD plasma is hotter, yet nonequilibrium (1900 K). Electronic excitation temperature (9800 K) and OH concentration (3×10^{16} cm^{-3}) were estimated in GD assuming the Boltzmann distribution of excited states [5].

OES characteristics showed that electrons with the highest energies are present in TS. These electrons initiate dissociations, ionizations and excitations of various species, leading to O, N, H, OH radicals, and N_2^+ ions. O radicals may react with air O_2 and form ozone O_3. These results synthesized with those of sterilization indicate that the role of radicals and other active species is very important.

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 amount of OH radicals forming by dissociation of the vaporized water. Streamer corona was the least efficient in the static regime. This is partly because it was the least energetic and partly because the active region was only in the proximity of the needle tip.

3.3. Flowing regime treatment in the discharge tube

Sterilization of water contaminated by *S. typhimurium* in positive TS in the 5-discharge tube in the flowing regime gave better results than in the static regime. We treated 0.1 l of a contaminated water or a physiologic solution and varied the treatment times (15-28 min), i.e. flow rates (3-6 ml/min). The typical discharge parameters were: total mean current 5 mA, $f = 6$ kHz. The decontamination efficiencies reached 99.25-99.99%, which is by 2-3 orders of magnitude larger than in the static regime, despite the residence time of the treated water in the discharge zone was shorter (10-20 s). This can be explained by much better 'volume efficiency,' i.e. the portion of water volume directly treated by the discharge was substantially larger here than in the static dish. The corresponding D-values were also substantially shorter (2.8-8.1 s).
The plasma treated water (or physiological solution) had slightly increased temperature (from 22 to 31 ºC), conductivity (from 0.52 to 0.8-1.2 mS/cm for water and 15.2 to 16.4 mS/cm for physiologic solution), and decreased pH (from 7.4 to 3). The temperature increase is negligible from the point of view of bacterial survival, but the effects of increased conductivity and especially reduced pH may be important. They will be subjected to further analyses. Similar pH decrease and conductivity increase were reported by several authors performing discharges with water, e.g. [7-8].

3.4. Flowing regime treatment through the high voltage electrode

The sterilization of *S. typhimurium* by corona discharge was very weak in the static regime. This was due to very small ratio of discharge active region to the total volume of the reactor and of the treated volume. We improved this 'volume efficiency' by flowing the treated medium directly through the corona active region in the proximity of the needle electrode. The electrostatic spaying through the corona active zone contributed to the improved efficiency of sterilization.
The achieved sterilization efficiencies in positive streamer corona in this arrangement were up to 89 % at mean power of 0.14 W. Transient spark gave up to 96 % at higher mean power of 1.22 W. Despite The sterilization rate is very fast: the calculated D-values are 0.2 s for SC and 0.13 s for TS.

3.5. Identification of mechanisms: TBARS method

Interaction of the reactive oxygen species with the biological membrane of cells results in the peroxidation of membrane lipids. The final product of lipoperoxidation is malondialdehyde (MDA). Its reaction with thiobarbituric acid (TBA) at temperature 90–100 °C gives a coloured product with the absorbtion maximum at 532 nm, which can be quantified spectrophotometrically [9-10].

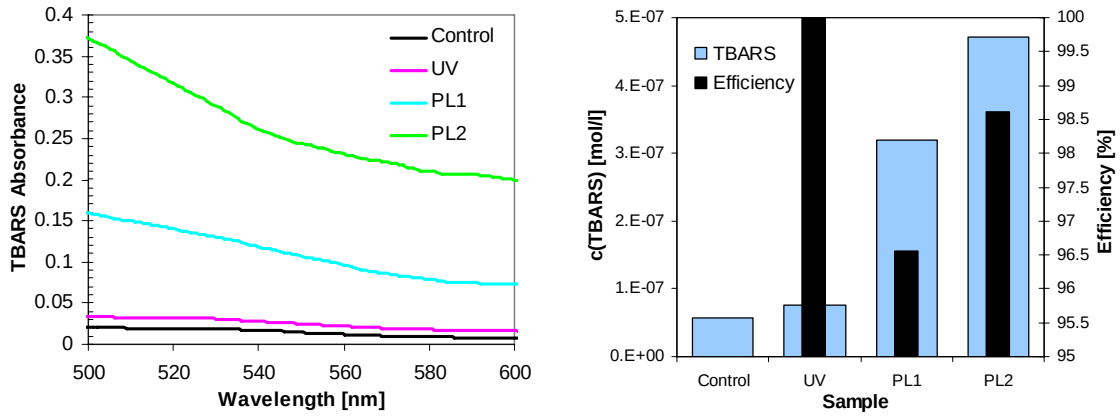


FIGURE 5. TBARS absorption spectra of *S. typhimurium* and measured concentrations of TBARS together with the inactivation efficiency.

This method of the *thiobarbituric acid reactive substances (TBARS)* was applied for the measurement of the oxidative stress induced *S. typhimurium* [11] after exposure to plasma (physiologic solution flowing through three TS discharges at two flow rates 1.44 ml/min (PL_1) and 0.5 ml/min (PL_2)) and 2-minute UV radiation by Hg lamp in comparison to the control – unexposed bacteria. We assigned the TBARS concentrations from the absorption spectrum as differences of absorbances at 532 (MDA) and 580 nm (background) from Lambert-Beer's law with the absorption coefficient 1.57×10^5 $mol^{-1}.l.cm^{-1}$. The corresponding absorption spectra and the measured TBARS concentrations are shown together with the inactivation efficiencies in Fig. 5. UV radiation resulted in low TBARS concentration despite the efficiency was 100 % (everything killed). This indicates that UV radiation is a very effective sterilization tool but its dominant mechanism is not oxidation of membranes. Both plasma exposed samples resulted in significant TBARS concentrations with efficiencies of 96.6 and 98.6 %, indicating that oxidations of bacterial cells are important inactivation mechanisms in the plasma. More reactive oxygen species leads to higher efficiency. This result correlates well with that revealed by the OES.

3.6. Sterilization of spores on surfaces

Sterilization of *B. cereus* spores on plastic and paper surfaces by negative corona discharge was tested. The spores were prepared from the live bacteria in the physiological solution and 50 μl of the spore suspension was then dropped on sterile plastic or paper plates and dried. The achieved spore inactivation efficiency on plastic foil after 5 min exposure to negative corona was 98 %, with the corresponding D-value of ~3 min. Lower efficiency was obtained on paper: 86 % with D ~6 min.

4. CONCLUSIONS

We investigated sterilization of water contaminated by bacteria (*S. typhimurium* and *B. cereus*) by three types of DC electrical discharges in atmospheric pressure air with one electrode submerged in water: streamer corona and two novel types: transient spark and glow discharge. The discharges generate non-thermal plasmas with various gas temperatures and properties. Satisfactory sterilization of water was obtained in the static regime, with the highest efficiency in the transient spark. In the flowing regime treatment by 5 parallel transient sparks, higher decontamination efficiencies were achieved in shorter treatment times. High efficiencies and short treatment times were also obtained in corona or transient spark when the treated water flew through the active plasma region. We successfully tested sterilization of plastic or paper surfaces contaminated by *B. cereus* spores in negative corona. Spectroscopic investigations and TBARS method indicated important bio-inactivation mechanisms, especially the major role of radicals and oxidative species.

ACKNOWLEDGEMENT

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