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DC discharges in atmospheric air for bio-decontamination – spectroscopic methods for mechanism identification

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Abstract. Three types of DC electrical discharges in atmospheric air (streamer corona, transient spark and glow discharge) were tested for bio-decontamination of bacteria and yeasts in water solution, and spores on surfaces. Static vs. flowing 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 inactivated by negative corona. The microbes were handled and their population evaluated by standard microbiology cultivation procedures. The emission spectroscopy of the discharges and TBARS (thiobarbituric acid reactive substances) absorption spectrometric detection of the products of lipid peroxidation of bacterial cell membranes indicated a major role of radicals and reactive oxygen species among the bio-decontamination mechanisms.

PACS. 52.80.Hc Glow; corona – 52.80.Tn Other gas discharges – 52.70.Kz Optical – 87.64.-t Spectroscopic and microscopic techniques in biophysics and medical physics

1 Introduction

Atmospheric pressure non-thermal plasmas are nowadays widely investigated for various biological and bio-medical applications. Sterilization and bio-decontamination by atmospheric pressure plasmas generated by corona [1], dielectric barrier [2,3], radio-frequency [4–6], microwave [7] discharges, and plasma jets and afterglows [2,5,8–11] have been studied by these and many other researchers in various gases, either in direct or in remote exposure to plasma, tested on various bacteria, spores, fungi and viruses, reaching from partial disinfection (1–2 log reduction of microbial population) up to complete sterilization (>5 log reduction or everything killed). In bio-decontamination, it is very important to assess the role of various mechanisms involved. A few works provide an insight and discuss these mechanisms involved in atmospheric pressure plasmas and stress the major role of radicals [2,10–13], whereas some find dominant UV radiation [5], or charged particles [14]. A few works go beyond sterilization and deal also with the diagnostics and inactivation of endotoxins and pyrogens released from the cells after plasma treatment, e.g. nucleic acids and proteins [8] and lipopolysaccharides [7].

We investigate three types of DC discharges in atmospheric air and test their bio-decontamination effects on selected bacteria and yeasts in water solutions and spores on dielectric surfaces. Bio-decontamination of water is important from the viewpoint of waste water cleaning or drinking water disinfection. Spore-contaminated plastic surfaces were chosen to mimic sterilization of heat-sensitive plastic materials used in medicine. Paper surfaces can be potentially used as carriers of bio-terrorism agents (e.g. anthrax contaminated letters) or are susceptible to mold degradation in archives and libraries. Bio-decontamination of molded paper in dielectric barrier discharge was tested by [15].

We identify the dominant mechanisms by comparing the electrical characteristics of the investigated discharges, their emission spectra, bio-decontamination effects in various discharge and flow regimes, and by TBARS detection of oxidative stress of microbial cells.

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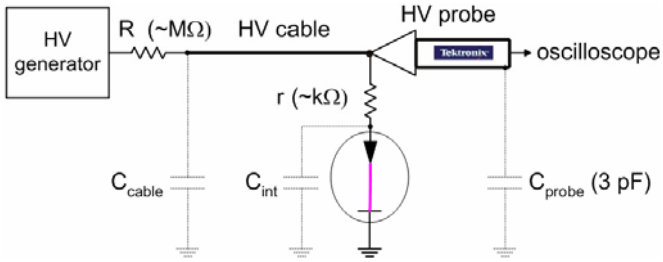

Fig. 1. (Color online) Schematic of the electrical circuit of DC discharges.

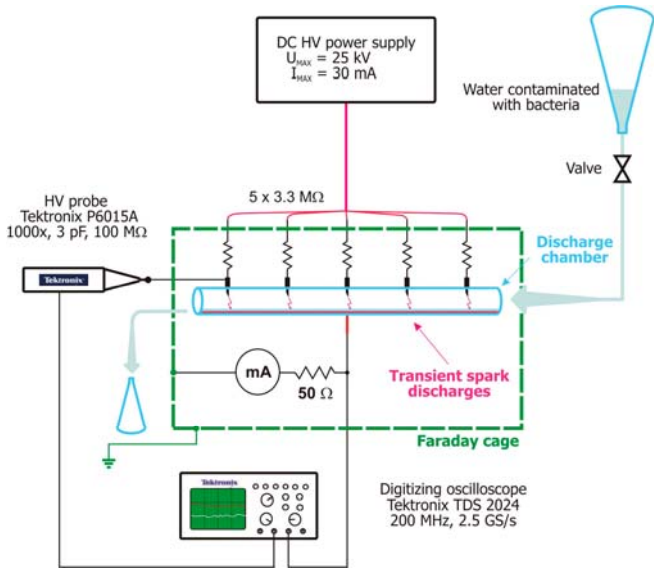

Fig. 2. (Color online) Experimental set-up and a discharge tube with 5 parallel discharges for flowing regime water treatment.

The plasmas generated by the studied DC discharges induce chemical effects important for bio-decontamination, as well as for environmental applications, such as VOC abatement that we successfully tested in the past [16,17].

2 Experiment

2.1 Experimental setups

The experimental setup for fundamental investigations of the DC discharges was shown elsewhere [18,19]. A simplified electrical scheme is depicted in Figure 1. A DC high voltage was applied through the ballast resistor R ($\sim$MΩ) and through the high voltage cable on to the needle electrode. The bio-decontamination effects of the DC discharges were tested in both static and flowing regimes. In the static regime, the discharges were operated in point-to-plane geometry, with a high voltage needle electrode and a plane electrode submerged in a certain volume (typically 5 mL) of contaminated water in a Petri dish.

Figure 2 shows the setup for the continual flowing water treatment. Five (or three) parallel discharges were operated in a glass (or plexi) 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.

Fig. 3. (Color online) Flowing the contaminated water through the high voltage needle electrode and electro-spraying effect in the corona discharge.

Another flowing regime setup was developed especially to increase the inactivation efficiency of corona discharge. It enabled flowing the treated medium (contaminated water) directly through the high voltage needle electrode, and so through the corona active region in its proximity. The effect of electrostatic spraying occurred when the high voltage was applied on the needle electrode. Figure 3 shows the set-up and demonstrates the water spraying effect through the corona active zone. This setup was also used for other discharges (transient spark). We also used point-to-plane electrode geometry for the bio-decontamination 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 a negative corona was applied for 5 or 10 min. The treated surfaces were placed on the grounded plane electrode.

The discharge voltage 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, or the total mean DC current in 5-discharge tube with a milliamp meter. 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).

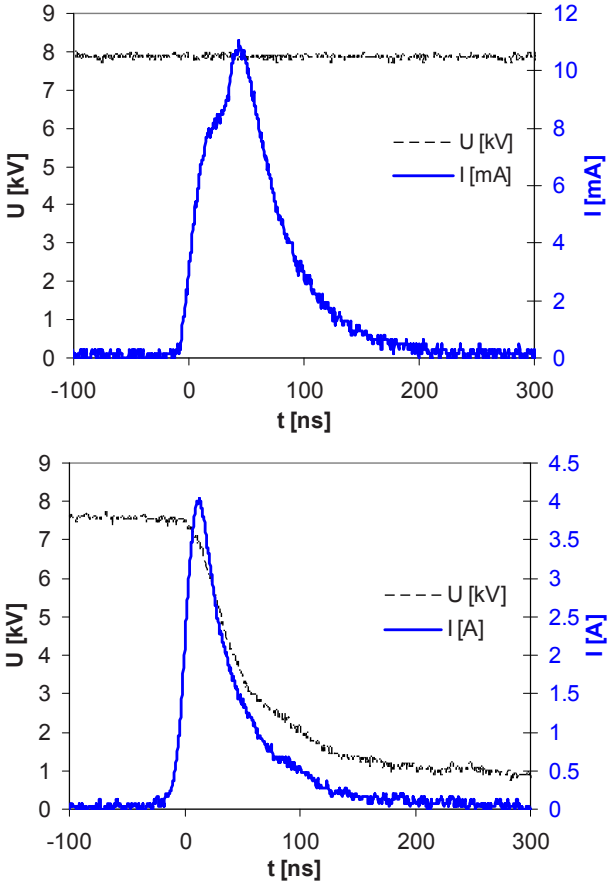

Fig. 4. (Color online) Streamer corona (a) and transient spark (b) voltage and current pulse waveforms in positive needle water – gap of 6 mm, $R = 3.5$ MΩ SC: 14 kHz, $I_{max} = 12$ mA, $U = 8$ kV, TS: 1.2 kHz, $I_{max} = 4$ A, $U = 7.5$ kV.

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 (SC), 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 documented in detail in our previous work [18].

The discharge typical voltage and current waveforms are shown in Figure 4. When a high voltage of a few kV is applied to the point electrode, *streamer corona* appears. SC is typical with small current pulses of streamers (∼10 mA) with a repetitive frequency of 10–30 kHz, during which the discharge voltage remains fairly constant [20] and generates very cold plasma (∼300 K).

As the voltage is further increased (to ∼8 kV in 6 mm gap), the streamers establish a conductive channel that leads to a spark formation. During the streamer-to-spark transition the local gas heating in the streamer-induced channel decreases the gas density N, thus enhancing the reduced electric field E/N. Since E/N is the main pa-

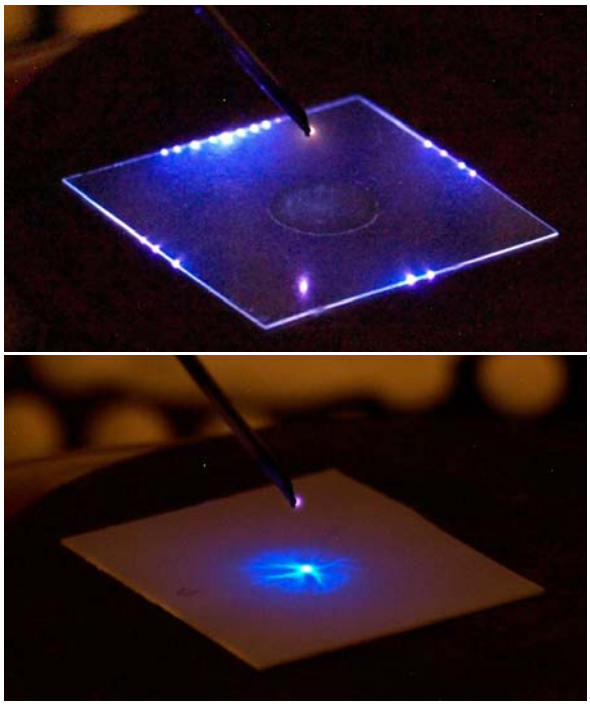

Fig. 5. (Color online) Photos of the negative corona on plastic foil (a) and paper (b) surfaces, gap distance 4 mm.

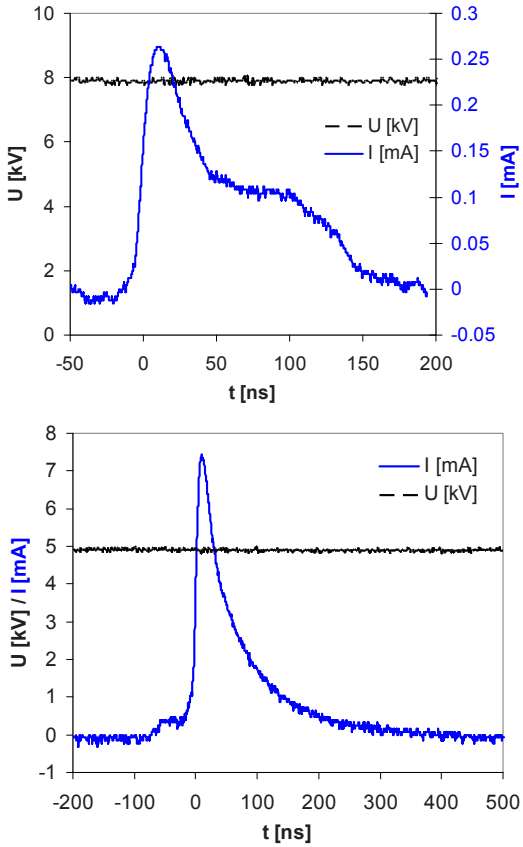

Fig. 6. (Color online) Negative corona typical voltage and current pulse waveforms with plastic foil (upper panel, $f = 2.7$ MHz) and paper (lower panel, $f = 1.3$ kHz) surfaces on the grounded electrode, gap distance 4 mm.

rameter controlling the rate of electron-impact reactions, especially ionization, this state leads to an enhanced ionization, resulting in a spark breakdown with excessive current pulse [21]. In our case, the spark pulse current is limited by (1) the ballast resistor R that drops the voltage as the current increases, and (2) the capacity C between the electrodes that is small (order of 10 pF). Thus, even if the sparks forms, it is only transient since the discharged energy is small (0.1–1 mJ). After the pulse, C is recharged by a growing potential on the stressed electrode. As soon as C is charged enough again, it triggers a new pulse. This *transient spark* becomes then a repetitive streamer-to-spark transition discharge, with each spark pulse (∼1 A) preceded by one or a sequence of streamer pulses. The repetitive frequency of pulses is 0.5–5 kHz, and increases with the growing applied voltage. Thanks to the very short pulse duration (∼10–100 ns) given by the small C and a limiting R, the plasma cannot reach LTE conditions and remains at relatively low gas temperature, depending on frequency, i.e. dissipated power (∼500–1500 K).

By setting the values of R and C we can either obtain the TS pulses and control their amplitude and frequency, or obtain a continuous discharge. This pulse-less discharge typically appears after the spark pulse if $R \leq 2$ MΩ and $f > 5$ kHz, and so the voltage drops to a certain small value but not zero, high enough though to sustain a small current (∼mA). It has a character of a *glow discharge*, and is typical with constant voltage and current (∼1–10 mA), a cathode fall of several hundreds V, and a luminous positive column that occupies most of the gap space. Its current-voltage characteristic is descending and provides relatively hot (1500–2000 K) yet non-thermal plasma. GD was in more detail described in [19,22].

The *negative corona* was employed for bio-decontamination of spores on surfaces. The discharge appearance on plastic foil and paper surfaces is shown in Figure 5. The negative corona above plastic foil demonstrated typical Trichel pulses [20] with frequencies 0.5–5 MHz and amplitudes up to 0.26 mA, as shown in Figure 6. The frequency of pulses increased with the applied voltage, up to a certain limit when the pulsed mode transited into a pulse-less glow mode of constant low current (0.12–0.27 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 [23] that forms on resistive porous layers covering the low voltage electrode. The corresponding discharge pulses were higher than Trichel pulses (up to 16 mA) but with lower frequency (∼1–2 kHz), as shown in Figure 6.

2.3 Treated microorganisms

Bio-decontamination effects of investigated DC discharges were tested on selected bacteria and yeasts in water or physiologic solution with initial populations from 10^3 up to 10^7 colony forming units per mL (CFU/mL). Spores in physiologic solution were dropped on the surfaces (plastic foil, paper), dried, and then treated by corona discharge.

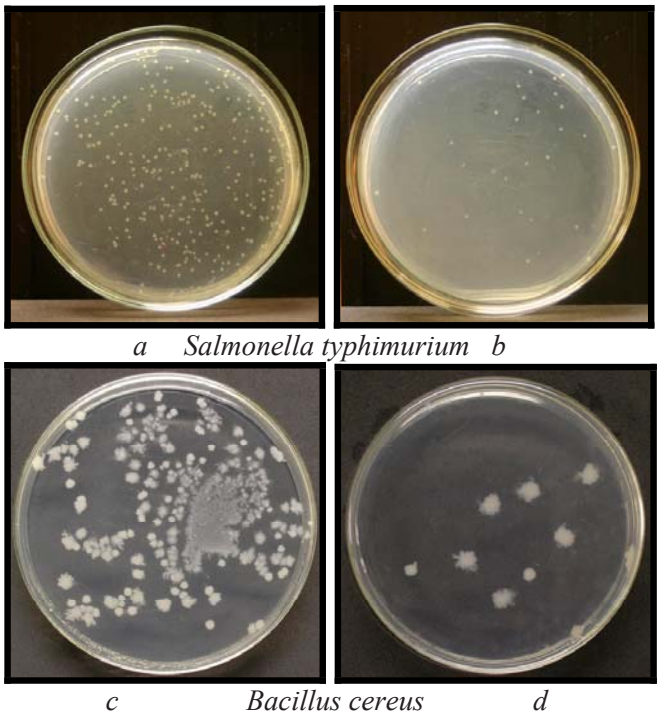

Fig. 7. (Color online) Cultivated bacteria on Petri dishes. Reference ((a),(c)) and after-treatment ((b),(d)) samples.

The bacteria cultivated on Petri dishes are illustrated in Figure 7. The following microorganisms were treated:

1) *Salmonella typhimurium* (*Salmonella enterica*, serovar Typhimurium), Gram-negative bacteria (strain TA 98): pathogen causing salmonelosis diseases; its inactivation is important from the viewpoint of drinking water decontamination.
2) *Bacillus cereus*, Gram-positive bacteria (and spores): belongs to the same group as extremely hazardous *B. anthracis* (Anthrax precursor), which nowadays represents one of the highest bio-terrorism risks.
3) *Saccharomyces cerevisiae*, yeast (strain CML282, provided kindly by Enrique Herrero, Universitat de Lleida, Spain), represents traditional eukaryotic model organism. Although *S. cerevisiae* are not pathogenic to humans, other yeasts, e.g. genus *Candida*, are opportunistic pathogens that can cause infections mostly in immunocompromised people. Its inactivation is important in medical instruments sterilization.

2.4 Microbial handling and cultivation procedure

The experiments of microbial bio-decontamination were carried out in a box with exhaust ventilation and a UV germicide lamp to provide sterile environment, working above natural gas flame to ensure the sterility of all objects, vessels, and instruments used during the manipulation with microorganisms. The microbial cultivation was performed in the following steps: we first prepared an overnight bacterial culture in a sterile liquid nutrient, in a

Table 1. Parameters of the static treatment of *S. typhimurium* in water in point-to-submerged plane geometry.

Discharge (polarity)	T_g (K)	Active species	Concentration (CFU/mL)	P (W)	Efficiency (%)	Log reduction	D-value (s)	E-value (J/mL log)
SC (+)	300–400	N_2*(C,B), OH	7310	0.4	22.98	0.11	529	42.3
(–)			6860	2.5	6.85	0.03	1947	973.3
TS (+)	450–650	N_2*(C,B), OH	6350	4	71.65	0.55	110	87.7
(+)		N_2^+, N, O, H	23 770	3.4	50.36	0.30	197	134.2
(–)			7420	7.2	34.5	0.18	326	470.2
(–)			27 080	4.8	55.83	0.35	169	162.3
GD (+)	1800–2000	N_2*(C,B), OH	25 460	10	55.18	0.35	172	344.3
(–)		NO	26 330	10	43.83	0.25	240	479.1

shaker. We also prepared a sterile solid nourishing medium – agar, and poured it into sterile Petri dishes, on which the bacteria were grown. Cultivated bacteria in the liquid nutrient were compared with McFarland turbidity scale to assess their initial population per mL. They were then diluted in physiologic solution or demineralized or even tap water to obtain desired concentrations, typically 10^3–10^4 CFU/mL, in some experiments higher. The plasma experiments were performed with various discharge types, parameters and treatment times. Usually 20–50 μL of plasma treated and reference (control) samples were spread on Petri dishes with agar. We took 3–5 Petri dishes from each sample for statistical evaluation. These were incubated during 12–24 h in a thermostat at 37 °C. The grown CFUs were counted by a CFU counter. The treated and reference samples were finally evaluated to obtain the survival curves and inactivation efficiencies.

Yeasts were grown in liquid synthetic complete media (CSM) with 2% glucose or 2% raffinose as a carbon source. After plasma treatment, treated and nontreated (control) cells suspensions were plated on solid CSM media and cultivated at 28 °C. After 3–4 days, the numbers of colonies were counted and survival rates evaluated.

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 for 15, 30, and 60 s treatment times are shown in Figure 8. The graph shows 2 experimental sets, starting at 7000 and 26 000 CFU/mL. The number of CFUs decreased with the treatment time in all discharges. We express the relative microbial population decrease, i.e. the inactivation efficiency. The typical discharge parameters are listed in Table 1. The highest efficiencies were obtained in the positive TS with 60 s treatment time, the lowest in the coronas; GD gave fairly high efficiencies as well but at higher energy costs, due to the gas heating. SC was the least efficient, partly because it was the least energetic and partly because the active region was only in the proximity of the needle tip.

Conventional sterilization methods are often evaluated by the *D-value* (decimal value) that represents the time

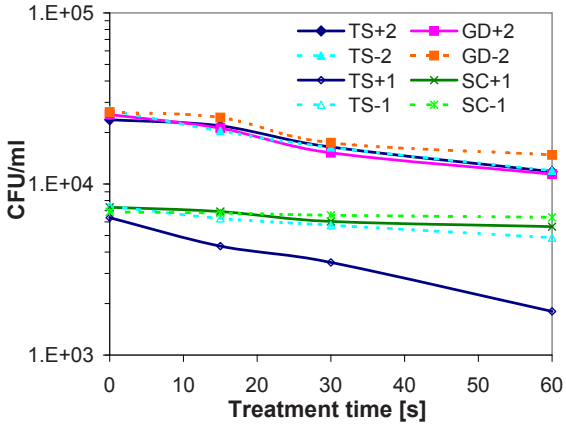

Fig. 8. (Color online) *S. typhimurium* survival curves in semi-logarithmic scale. +: positive, –: negative polarity.

of the microbial population reduction by 90% (1 log). We introduce a new parameter: E-value (Joule per treated volume and one log reduction) to express the combined energy requirements and efficiency of the process. The achieved D-values varied from 1.8 (TS) to 32 min (SC) and E-values from 42 (SC) to 479 J/mL log (GD). We also tested the inactivation of the spore-forming *B. cereus* in the static regime with efficiency 98.7% obtained after 60 s treatment in positive TS.

The reached efficiencies ($\leq$1 log reduction) in the static treatment are of course not sufficient for bio-decontamination, we can speak just of a partial disinfection. This is because the plasma volume was very small compared to the treaded water volume and there was no mixing of the water, so only a small part of its surface below the discharge was efficiently treated. However, these tests, along with the emission spectroscopic analysis, were aimed at comparing the effects of the three discharge types on bacteria.

3.2 Flowing regime treatment in the discharge tube

Bio-decontamination of *S. typhimurium* by positive TS in the 5-discharge tube in flowing regime gave better results than in the static regime. We treated 0.1 l of a contaminated water or a physiologic solution (initial population $\sim 10^3/10^4$ CFU/mL) and varied the treatment times (15–28 min), i.e. flow rates (3–6 mL/min).

Table 2. Parameters of the flowing treatment of *S. typhimurium* and *S. cerevisiae* in water in 5-discharge tube and through the high voltage needle electrode.

Setup	Discharge (polarity)	Microorganism	Concentration (CFU/mL)	P (W)	Efficiency (%)	Log reduction	D-value (s)	E-value (J/mL log)
5-discharge tube	5 TS (+)	*S. typhimurium*	12450	35	99.99	4.10	2.77	92.3
	5 TS (+)	*S. typhimurium*	6650	39	99.7	2.52	3.75	139.2
	5 TS (+)	*S. typhimurium*	61850000	40	93.21	1.17	31.28	595.8
	3 TS (–)	*S. typhimurium*	12500000	18	97.6	1.62	27.01	463.0
	3 TS (–)	*S. typhimurium*	11900000	18	98.61	1.86	67.81	1162.5
Through HV needle electrode	SC (+)	*S. typhimurium*	14800000	0.01	93.24	1.17	0.13	0.7
	SC (+)	*S. typhimurium*	22280000	0.02	83.45	0.78	0.38	2.6
	SC (+)	*S. typhimurium*	54466667	0.12	80.58	0.71	0.41	20.2
	SC (+)	*S. cerevisiae*	2500000	0.01	54.62	0.34	0.6	2.8
	SC (+)	*S. cerevisiae*	2500000	0.26	75.25	0.61	0.49	51.3
	SC (–)	*S. typhimurium*	59733333	0.25	79.31	0.68	0.43	43.5
	SC (–)	*S. typhimurium*	9575000	0.45	67.7	0.49	0.6	110.0
	TS (+)	*S. typhimurium*	48466667	1.44	93.21	1.17	0.25	147.9
	TS (+)	*S. typhimurium*	14800000	2.33	93.51	1.19	0.12	234.9
	TS (+)	*S. typhimurium*	1175000	1.33	96.6	1.47	0.2	108.7
	TS (+)	*S. cerevisiae*	2500000	1.9	100	7.00	0.04	32.6
	TS (+)	*S. cerevisiae*	2500000	2.85	94.8	1.28	0.23	266.4
	TS (–)	*S. typhimurium*	62600000	0.28	79.18	0.68	0.43	49.3
	TS (–)	*S. typhimurium*	9575000	0.14	69.83	0.52	0.57	32.3

The typical discharge and bio-decontamination parameters are in the Table 2. The reached inactivation efficiencies (99.25–99.99%) are by 2–3 logs 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 (2.8–31 s).

The treated water (or physiological solution) had slightly increased temperature (from 22 to 31 °C), conductivity (from 0.52 to 0.8–1.2 mS/cm for water and from 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 and explained by several authors performing discharges with water, e.g. [24–26]. No significant effect of the medium (water vs. physiologic solution) was observed.

Three parallel negative TS discharges in the same discharge tube were also tested in the flowing regime but with much higher initial populations and slower flow rates. The obtained efficiencies reached up to 98.6% with D-values of 27–68 s.

3.3 Transient spark pulse shape effect

With the TS discharge, we explored the effect of the pulse shape. As mentioned earlier in Section 2.2, the TS pulse amplitude and duration are given by the repetitively discharging internal capacity of the chamber (C_{int} ∼ 1–10 pF). When a high voltage probe and a high voltage cable were used, they added their own capacities to this C_{int}. To prevent this effect, we separated the discharge chamber from the high voltage probe and cables by a small resistor r in order to minimize the capacity discharging in the spark pulse (see Fig. 1). We tested $r = 0$ (no resistor), 510 Ω, and 1.5 kΩ. Of course, a correction of the measured voltage on this r was then done. The pulse shape changed dramatically with various r tested, as demonstrated in Figure 9. With increasing r, the pulse amplitude decreased but its duration extended. The amplitude decrease was due to the lowering capacities additional to C_{int}, and the duration

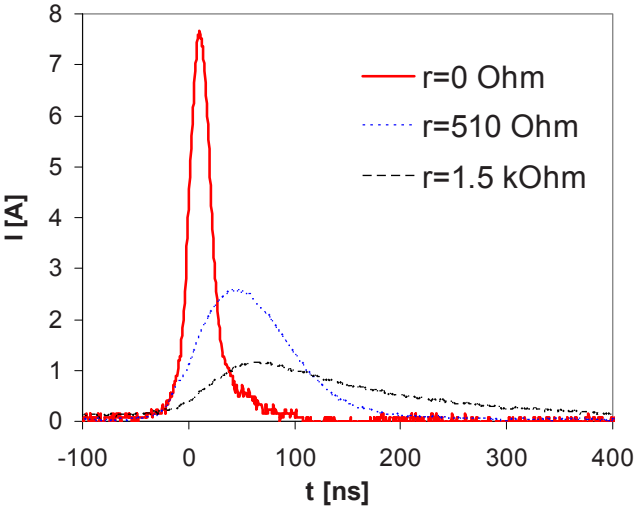

Fig. 9. (Color online) Effect of the separating resistor r on the TS current pulse.

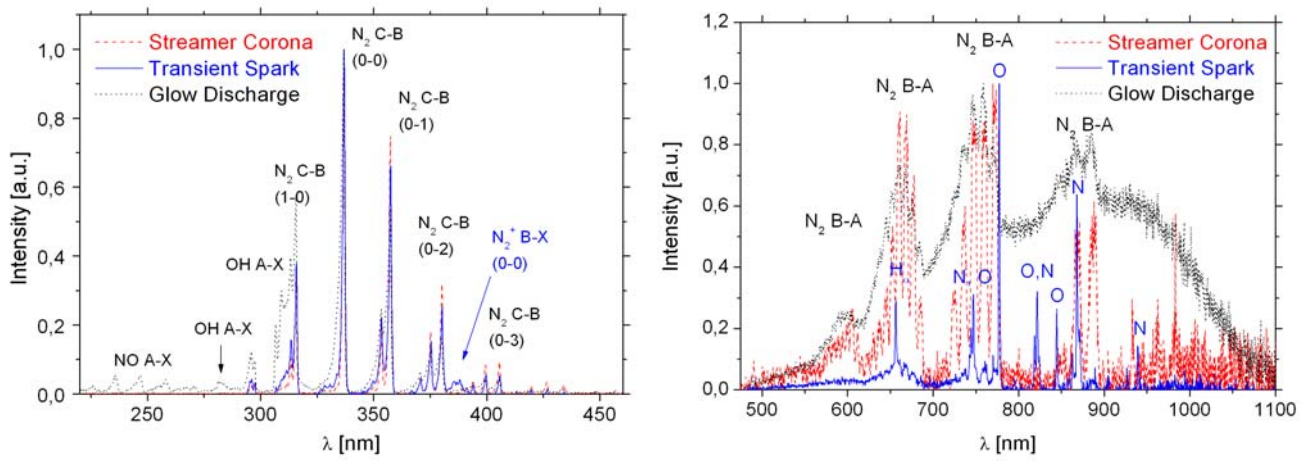

Fig. 10. (Color online) Typical emission spectra of DC discharges in UV and VIS-NIR regions. Gap: 4 mm; SC: 26 kHz, $I_{max} = 25$ mA; TS: 1 kHz, $I_{max} = 1.5$ A; GD: $I = 6$ mA.

extension due to the larger time constant. The mean current was kept approximately constant. Interestingly, the bio-decontamination tests with various r applied showed that substantially higher efficiencies were obtained with no r, i.e. strong and short pulses (∼8 A, ∼50 ns). Such pulses result in strong plasma nonequilibrium and generation of radicals and other active species, and low energy losses by gas heating. Similar effect was observed when comparing the electrical properties, the emission spectra and the bio-decontamination effects of TS and GD, as described in Section 3.2.

3.4 Flowing regime treatment through the high voltage electrode

The bio-decontamination 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 to the treated volume of microbial medium. We improved this volume efficiency by a flow of the treated medium directly through the corona active region in the proximity of the needle electrode. This idea was first introduced for polluted gas treatment through the high voltage needle electrodes [27]. The electrostatic spaying of contaminated water through the corona active zone contributed to the improved efficiency of bio-decontamination. The typical parameters and achieved inactivation results in both positive and negative streamer corona and transient spark with contaminated water flowing through the active zone are listed in Table 2. Despite the microbial population decrease was not by several logs, it was very fast and low-energetic: the calculated D-values are as short as 0.12 s and E-values from 0.72 J/mL log. These D-values were referred to the active residence time of the medium between the electrodes. Further improvements of this setup with electro-spray will expectantly enhance the inactivation at very low energy costs.

3.5 Bio-decontamination of spores on surfaces

Bio-decontamination of *B. cereus* spores on plastic and paper surfaces was tested by negative corona discharge. 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 stain on the surface contained 10^4–10^5 spores. As described previously in Section 2.2, the discharge operation was different when operated above plastic foil and paper. Typical achieved bio-decontamination efficiencies of spores on plastic foil surfaces after 5 min exposure to negative corona were 98.3%, with the corresponding D-value ∼3 min and mean power of 2.1 W. Significant bio-decontamination was observed with high-frequency Trichel pulses or in the pulse-less mode.

Slightly lower efficiency was obtained on paper surfaces: 85.9% with D-value ∼6 min but lower $P = 0.11$ W. It was technically impossible to increase the pulse frequency (and so the power) in this case, because the corona easily transited to the spark when the applied voltage increased. When the spark occurred, it burned the hole in the paper and so established a direct conductive passage to the grounded electrode. Since then, the discharge became constricted to the hole only.

3.6 Identification of mechanisms: optical emission spectroscopy (OES)

OES 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 gives insight in ongoing plasma chemistry [19,28]. Here, we employ OES, together with the comparison of the bio-effects of the three discharges to identify the dominant mechanisms involved in bio-decontamination. The typical UV and VIS-NIR spectra are shown in Figure 10.

Rotational, i.e. gas, and vibrational temperatures are evaluated by fitting experimental with simulated spectra using program SPECAIR [29]. SC and TS generate cold, nonequilibrium plasmas (300–550 K), GD plasma is hotter, yet nonequilibrium (1900 K).

OES characteristics of the applied discharges described in detail in [19] showed that electrons with the highest energies are present in TS. These electrons initiate dissociations, ionizations and excitations of various species. Atomic O, N and H radicals, and the N_2^+ ions have only been detected in TS, and there were a lot of OH radicals. O radicals may react with air O_2 and form ozone O_3. The advantage of GD, despite higher energy costs, is a large amount of OH radicals forming by dissociation of the vaporized water. These results synthesized with those of bacteria treatment indicate that the role of radicals and other active species is very important in bio-decontamination.

3.7 Identification of mechanisms: TBARS method absorption spectroscopy

Interaction of the reactive oxygen species generated in the plasma with the cell membranes results in the peroxidation of membrane lipids. The final product of lipoperoxidation is malondialdehyde (MDA), which can be quantified spectrophotometrically. The reaction with thiobarbituric acid (TBA) at high temperature (90–100 °C) gives a coloured product with the absorbing maximum at 532 nm [30,31].

This method of the thiobarbituric acid reactive substances (TBARS) was applied for the measurement of the oxidative stress induced in microorganisms [32] – bacteria and yeasts after exposure plasma and to UV B radiation for comparison. Samples and reagents used in TBARS measurement were prepared according to the recommended procedure [33]. We assigned the concentrations of the TBARS from the acquired absorption spectrum as differences of absorbances at 532 (absorption of MDA) and 580 nm (background) by using Lambert-Beer's law with the absorption coefficient of 1.57×10^5 mol^{-1} L cm^{-1} [34].

In experiment 1, medium with initial population $c_{01} = 11.9 \times 10^6$ CFU/mL of *S. typhimurium* flowing through three negative TS discharges in the discharge tube at two flow rates 1.44 mL/min (3 TS-*f*) and 0.5 mL/min (3 TS-*s*) and under 2 min UV radiation by Hg lamp in comparison to the control – unexposed bacteria in physiologic solution was investigated. In experiment 2, we treated medium with $c_{02} = 14.8 \times 10^6$ CFU/mL of *S. typhimurium* flowing through the high voltage electrode of positive TS and SC discharges at the flow rate 0.5 mL/min, and under 1 min UV radiation in comparison to the control.

The measured concentrations of TBARS together with the inactivation efficiencies for both experiments are shown in Figure 11. In both cases, UV radiation resulted in low TBARS concentration despite the bio-decontamination efficiency was 100% (everything killed) even with 1 min exposure. All plasma treated samples resulted in significant TBARS concentrations with efficiencies of 96.6 and 98.6% in 3 TS+discharges, and 93.5% in TS+ and 93.2% in SC+ flowing through the active plasma zone. This result indicates that UV radiation is a very effective bio-decontamination tool but its dominant mechanism is not peroxidation of cell membranes. On the other hand, significant TBARS concentrations in all plasma exposed samples indicate that oxidations of cell membranes by reactive oxygen species are important mechanisms of microbial inactivation in plasma. More reactive oxygen species generally leads to the higher efficiency. This result correlates well with the dominant mechanisms revealed by the OES and referred by other authors [2,10–13]: radicals and active species.

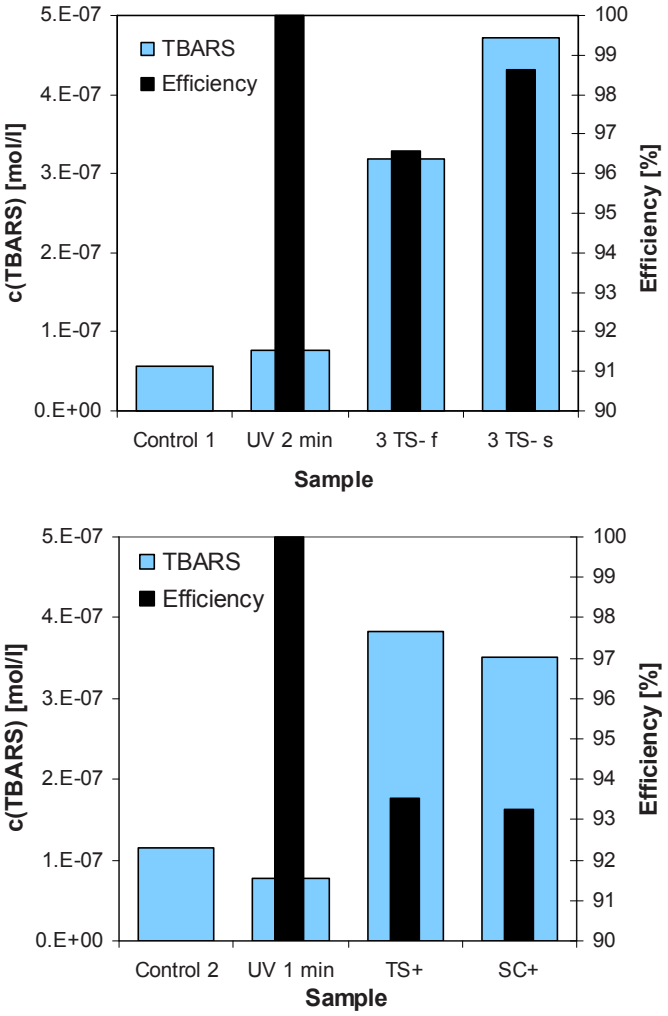

Fig. 11. (Color online) Measured concentrations of TBARS together with the inactivation efficiencies, (a): experiment 1 with 3 parallel negative TS discharges in flowing regime tube, initial population of *S. typhimurium* $c_{01} = 11.9 \times 10^6$ bacteria/mL, 3 TS-*f* denotes fast flow rate (1.44 mL/min), 3 TS-*s* slow (0.5 mL/min); (b): experiment 2 with positive TS and SC discharges with medium flowing through the high voltage needle electrode at flow rate 0.5 mL/min of *S. typhimurium*, $c_{02} = 14.8 \times 10^6$ bacteria/mL.

3.8 Conclusions

Three types of DC discharges in atmospheric pressure air in point-to-plane geometry or with one electrode submerged in water were investigated for bio-decontamination of water contaminated by bacteria and yeasts and of surfaces contaminated by spores. The streamer corona with small current pulses (∼10 mA) with a 10–30 kHz repetitive frequency generates very cold non-equilibrium plasma (300–350 K). With increasing applied voltage, the streamers transit to the transient spark: the spark with very short (<100 ns) current pulses (∼1 A) of 0.5–5 kHz repetitive frequency and very limited energy. Thanks to the very short spark pulse duration given by the small internal capacity of the discharge system and the limiting series resistor, the plasma cannot reach LTE conditions (500–1000 K). With an appropriate ballast resistor, this transient regime evolves into a pulse-less discharge, of properties of a typical glow discharge. All three DC discharges generate non-thermal plasmas with various gas temperatures and properties.

Bio-decontamination of water (or physiological solution) contaminated by microbes (*S. typhimurium*, *B. cereus*, and *S. cerevisiae*) was first tested in a non-optimized static regime, with the highest efficiency obtained in the transient spark with ultra short high current pulses. Glow discharge was efficient but too energetic. In the flowing regime treatment by 5 parallel transient sparks, much higher decontamination efficiencies were achieved in shorter treatment times and shorter D-values. Streamer corona and transient spark were found very efficient in the flowing regime with the treated medium passing directly through the high voltage needle electrode and thus through the active discharge zone: relatively high efficiencies at short treatment times resulted in very short D-values (0.12 s) and very low E-values (0.72 J/mL log). Electro-spraying effect occurring with corona in this regime enhanced the efficiency of the process. We also successfully tested bio-decontamination of plastic and paper surfaces contaminated by *B. cereus* spores in negative corona in 5 min treatment time. The bacteria, spores and yeasts were handled and their population evaluated by standard microbiology thermostatic growth cultivation procedures. In parallel, we are developing a rapid fluorescence spectroscopic method for bacterial live/dead population evaluation using SYTO 9 and propidium iodide fluorescent stains.

The emission spectra and the measured temperatures indicate that these discharges generate non-equilibrium plasmas with various excited species, molecular and atomic radicals. These investigations indicated important bio-inactivation mechanisms, mainly the major role of radicals and active species, generated especially in TS with high short pulses. The major role of reactive oxygen species in plasma treatment was confirmed by the absorption spectrometric detection of the products of microbial cell membrane peroxidation in TBARS (thiobarbituric acid reactive substances) method, where plasma exposure was compared with UV radiation. Comparing the effects of a direct vs. remote plasma exposure is envisaged in near future.

In summary, we demonstrated that non-equilibrium plasmas generated by three types of DC discharges in atmospheric air can be efficiently used for bio-decontamination of water and surfaces. The dominant microbial inactivation mechanisms in such plasmas are oxidations by radicals and active species.

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