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BIOCIDAL AGENTS IN BIO-DECONTAMINATION BY DC DISCHARGES IN ATMOSPHERIC AIR

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Bio-decontamination of water and surfaces contaminated by bacteria (Salmonella typhimurium, Bacillus cereus) was investigated in two types of positive DC discharges in atmospheric pressure air, in needle-to-plane geometry: the streamer corona and its transition to a novel regime transient spark with short high current pulses of limited energy. Both generate cold non-equilibrium plasma. Electro-spraying of the treated water through the needle electrode was applied for the first time and resulted in fast bio-decontamination. Experiments providing separation of various biocidal plasma agents, along with the emission spectra and coupled with oxidation stress measurements in the cell membranes helped better understanding of the mechanisms of microbial inactivation. The indirect exposure of contaminated surfaces to neutral active species was almost as efficient as the direct exposure to the plasma, whereas applying only UV radiation from the plasma had no biocidal effects. Radicals and reactive oxygen species were identified as dominant biocidal agents.

1. Introduction

Nonequilibrium plasmas, thanks to their reactive nature, find numerous biological and bio-medical applications, especially at atmospheric pressure for no need of costly vacuum equipment. Atmospheric pressure plasmas applied for sterilization and bio-decontamination are mostly generated by radio-frequency (RF) [1-2] discharges, and various plasma jets and afterglows [1-5], usually in rare gases (He or Ar) with/without admixtures of O_2 (or H_2O). They have been tested on a large variety of prokaryotic microorganisms (bacteria, spores, viruses) and some eukaryotic yeasts, fungi and microalgae, resulting in partial disinfection up to complete sterilization. Atmospheric air plasmas have additional advantages of no need of special gases and an easy application in ambient environment. Bio-decontamination by air plasmas was tested in DC [3, 6], dielectric barrier (DBD) [4, 7-10], RF [1], and pulsed discharges [4, 7]. The plasmas can be also generated directly in water or on water-air boundary [3, 8-9, 11], which is of great interest for water decontamination.

In bio-decontamination by plasma, it is crucial to understand the role of various mechanisms involved. The significant mechanisms depend on the plasma composition (gas), temperature, treated microorganisms and the environment (air, water, surfaces, etc.). In atmospheric pressure plasmas, the major role is typically attributed to radicals and reactive oxygen species (ROS, e.g. OH, O, O_3) [1, 2, 3-4, 6, 9-11] and to charged particles, especially O_2^- [7] affecting the cell membranes. UV radiation plays a role only if photons in UV C germicide region (220-280 nm) or in vacuum UV are produced [10-11]. In cold air discharges (corona, DBDs, pulsed discharges), NO γ and other sources of UV C or VUV are usually not generated, so radicals and ROS are identified as the dominant bio-inactivation agents [3-5, 8-9].

In this paper, the biocidal effects of two plasma sources in atmospheric air with water are investigated – positive DC *streamer corona* (SC) and a novel regime named *transient spark* (TS). Despite DC applied voltage, these discharges have a pulsed character with nanosecond repetitive pulses. We focus on the identification of the dominant plasma agents in bio-inactivation by coupling the electrical discharge characteristics, their emission spectra, and biocidal effects. Comparing direct with indirect plasma effects enables separation of various biocidal plasma agents. In addition, measurements of the oxidative stress induced in microbial cells applied for the first time in plasma bio-decontamination enable further indicate their respective roles.

2. Experiments

2.1 Experimental set-ups

The experimental setup for fundamental investigations of the DC discharges in point-to-plane geometry, with a high voltage (HV) hollow needle electrode enabling water flowing through the

discharge zone and a plane or mesh electrode is depicted in figure 1. The gap spacing was varied from 5-10 mm. A positive DC high voltage was applied through the ballast resistor *R* (20 MΩ for SC and ~5 MΩ for TS). The discharge voltage was measured by a high voltage probe Tektronix P6015A. The discharge current was measured: on a 50 Ω (SC) or 1 Ω (TS) resistor and by a Rogowski current monitor PEARSON 2877. The current and voltage signals were processed by a digitizing 200 MHz oscilloscope Tektronix TDS 2024. The discharges were photo- and video-documented with digital cameras Olympus E410 or Nikon Coolpix S10.

The emission spectroscopy optical system comprised a dual fibre-optic spectrometer Ocean Optics SD2000 for fast scanning in the UV and VIS-NIR regions (200-500 and 500-1050 nm, resolution 0.6–1.2 nm), fused silica lenses, and fibre optics. The discharge set-up was placed in a Faraday cage together with the optical components mounted on lateral and vertical translation stages.

The bio-decontamination effects of the DC discharges were tested on flowing water of ambient temperature. We also compared direct and indirect plasma effects on contaminated solid agar surfaces (Figure 2). A needle electrode was placed about 1 cm above the agar surface in the centre of the Petri dish and the discharge was applied for 1 or 2 min. In direct treatment, the agar was grounded with a wire. Indirect plasma effects on the contaminated agar were tested by:

1) placing the grounded mesh electrode ~2 mm above the agar, this shielded the electric field and trapped the charged particles, letting but neutral particles and partial UV light to reach the surface;
2) placing the 3 mm thick quartz window onto the agar surface and a grounded ring electrode on its top, this let only the light emitted from the discharge to reach the agar, including UV. We also tested MgF_2 window transmitting vacuum UV.

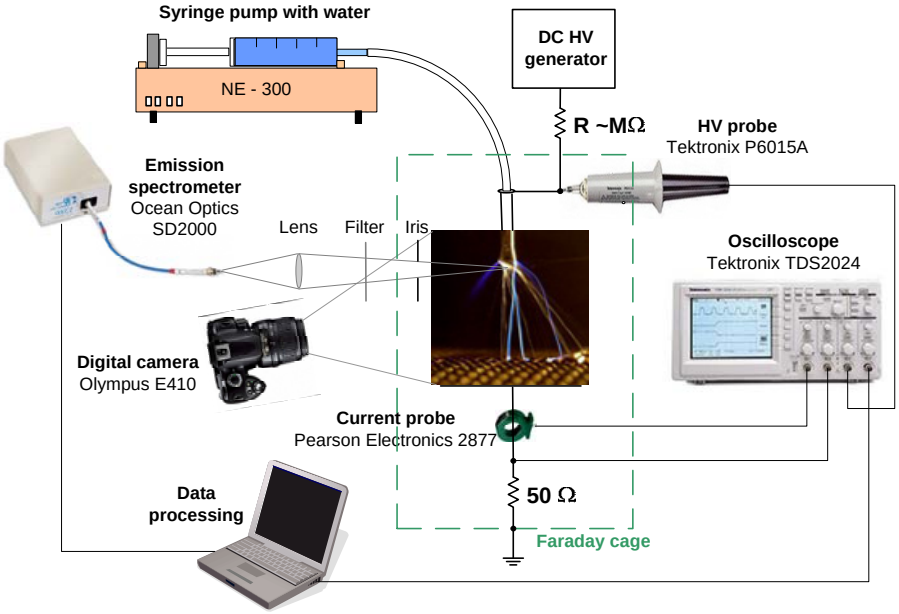

Fig. 1. Experimental set-up for DC discharges, with a high voltage hollow needle electrode enabling water flowing through the discharge zone and a plane or mesh electrode.

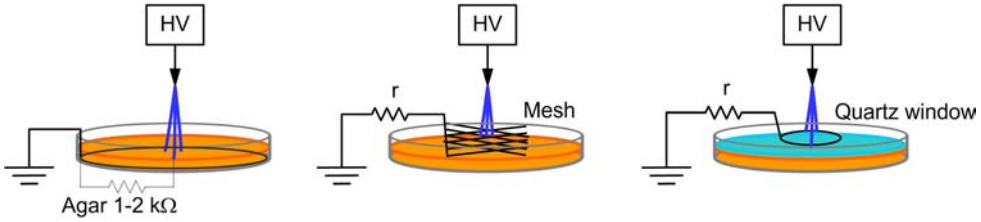

Fig. 2. Electrode arrangements for (a) direct and (b, c) indirect plasma treatment of contaminated agar plates. (b) Mesh electrode ~2 mm above agar surface trapped the charged particles and shielded the electric field. (c) Quartz (MgF_2) window only transmitted light from the discharge (including UV).

The agar in the direct treatment (Figure 2a) represents a certain electrical resistance, typically 1-2 kΩ depending on the water content. In indirect set-ups (Figure 2b,c), a small resistor *r* was inserted between the mesh (or ring) and the ground to simulate the agar's resistance. Its exact value was set empirically from case to case to make the discharge pulses of about the same amplitude and shape as in the direct treatment on agar. This ensured the same discharge properties in all three set-ups.

2.2. Treated microorganisms and microbial cultivation

Bio-decontamination effects of investigated DC discharges were tested on selected Gram-negative bacteria *Salmonella typhimurium* and Gram-positive *Bacillus cereus* in distilled water with initial populations 10^3 - 10^7 colony forming units per mL (CFU/mL), or directly spread on the solid nutrient (agar, Roth Ltd.) on a Petri dish, about 10^6 per dish. The microbial cultivation was carried out in a sterile environment. The plasma experiments were performed with both discharges, at various parameters and treatment times and repeated 5-8 times. 3–4 Petri dishes from each sample were taken for statistical evaluation. These were incubated during 12–24 h in a thermostat at 37 °C. The grown CFUs on the samples were counted and evaluated.

2.3. Measurements of the oxidative stress

Interaction of ROS with the bacterial cell membranes results in the peroxidation of membrane lipids. The final product of lipoperoxidation is malondialdehyde (MDA), quantifiable by spectrophotometry after the reaction with thiobarbituric acid (TBA) at 90–100 ◦C [12]. This method of *thiobarbituric acid reactive substances* (TBARS) was applied for the first time to measure the oxidative stress induced in bacteria in water exposed to SC and TS. We assigned the TBARS concentrations from the absorbance of MDA at 532 nm from Lambert-Beer's law with absorption coefficient 1.57×10^5 mol^{-1} L cm^{-1} [12].

3. Results and discussion

3.1. Applied DC discharges and their emission

Two types of positive DC discharges operating in atmospheric air with water were investigated: a well-known *streamer corona* (SC), and a novel *transient spark* (TS). These discharges generate non-equilibrium plasmas inducing various chemical and biological effects important in bio-decontamination. Their electrical parameters and emission spectra were documented in detail in our previous works [13-14]. SC is typical with small current pulses of streamers (~10 mA) with a repetitive frequency of ~10 kHz and generates cold plasma (~300 K).

With further voltage increase, the streamers establish a conductive channel that gradually leads to the spark pulse. However, when the sparks forms, it is in our set-up only transient since the discharged energy given by the external circuit is small (0.1–1 mJ). This *transient spark* is a repetitive (0.5–10 kHz) streamer-to spark transition discharge, with each spark pulse (~1 A) preceded by one or a sequence of streamer pulses. Thanks to the very short pulse duration (~10–100 ns) given by the small circuit capacity and a limiting series resistor *R*, the plasma cannot reach equilibrium conditions and remains at relatively low gas temperature, depending on frequency (~500–1500 K).

We employed optical emission spectroscopy, a powerful technique of plasma diagnostics to both SC and TS. They both generate cold, nonequilibrium plasmas (300–550 K) in the discharge channel. OES characteristics of the applied discharges described in detail in [13] showed that electrons with the highest energies were 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. Part of O radicals reacts with air O_2 and forms ozone O_3. There was no UV C radiation detected from SC and TS.

3.2. Flowing water treatment through the stressed electrode

The contaminated water flew directly through the stressed hollow needle electrode, and so through the plasma active zone in its proximity, which substantially improved the volume efficiency compared to our previous set-ups for water treatment [15]. The effect of electrostatic spraying (electro-hydro-dynamic atomization, EHDA) occurred when the high voltage was applied on the needle electrode [16]. The temperature of the treated water did not change in SC and was increased by maximum 10 K in TS. The lethal heat effect of the discharges to bacteria can be excluded.

3.3. Oxidative stress induced in bacteria

Figure 3 shows the TBARS concentration gain Δc(TBARS) correlated with the bio-decontamination efficiency of SC and TS applied to the electro-sprayed water with *S. typhimurium* and *B. cereus*. The same samples were irradiated by biocidal UV C radiation (Hg lamp, 254 nm, 1 min) for comparison.
UV C radiation induced almost no Δc(TBARS) despite its efficiency was very high. Obviously, UV dominant biocidal mechanism is not peroxidation of cell membranes. On the contrary, SC and TS treatments significantly enhanced Δc(TBARS). This indicates that oxidations of cell membranes by ROS are important in microbial inactivation. More ROS is linked with the higher efficiency.

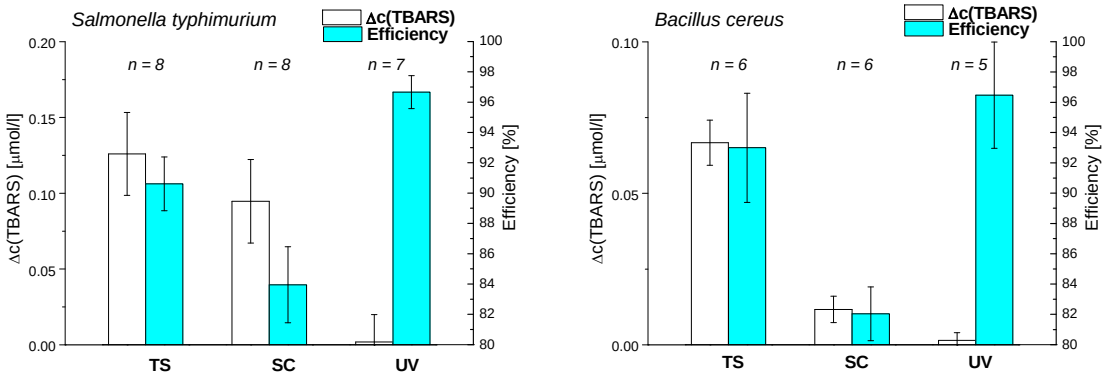

Fig. 3. TBARS concentration gains and decontamination efficiencies of *S. typhimurium* (left) and *B. cereus* (right) in water treated by SC and TS with electro-spray, compared with 1 min exposure to UV C (shown with standard error of the mean, *n* – number of repeated experiments).

3.4. Direct vs. indirect plasma treatment

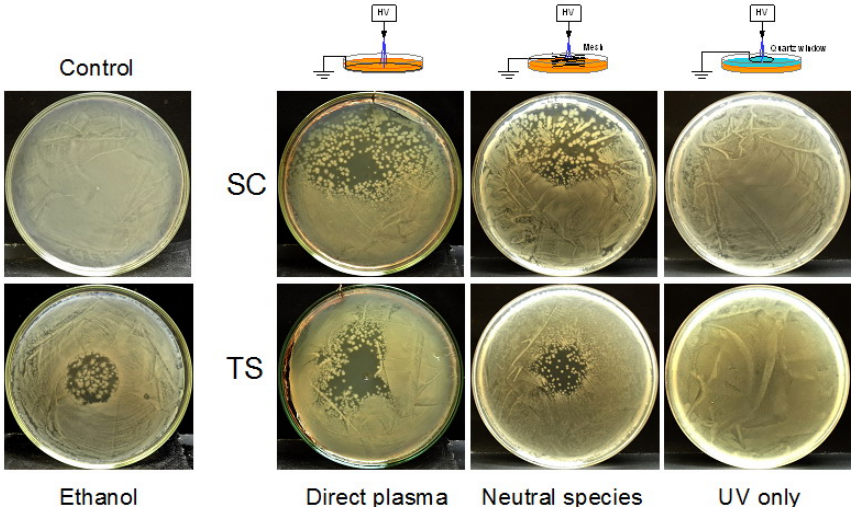

Fig. 4. Photographs of Petri dishes with contaminated agar by *S. typhimurium*; left side: untreated control and treated by ethanol, right side: treated by SC (upper row) and TS (lower row) direct, neutral species only, and UV light only. Results with *B. cereus* were similar.

We compared direct SC and TS plasma treatment with 2 types of indirect exposure described in section 2.1. Figure 6 shows the photographs of the Petri dishes with contaminated agar after direct and both indirect treatments, together with the untreated control sample, and a sample treated with 50 µL drop of liquid ethanol (96%) for comparison. The effects of plasma (and ethanol) on contaminated agar are clearly visible as dark voids, whereas control sample is homogeneously covered by cultivated bacteria (bright). Dark voids with bright spots represent incomplete decontamination, the spots are CFUs grown from single bacteria. With respect to the total number of bacteria spread on one Petri dish

(10^6), a few tens of survived bacteria that cause these bright CFUs in the voids are quite negligible.
Both direct plasma and indirect exposure to neutral reactive species (and partial UV) caused apparent bio-decontamination (voids) with on tested bacteria. SC resulted in a larger treated area on the Petri dish, likely due to the electric wind that drives active species from the point towards and along the agar surface in one preferential direction. TS treatment was localized in the dish centre but more intense.
Interestingly, there was very little difference between the direct and indirect plasma treatments with both discharges. This indicates that neutral reactive species are crucial even in the direct exposure. Exposure to the UV light only, transmitted by quartz or MgF_2 windows demonstrated no visible decontamination. This correlates with the emission spectra of SC and TS lacking any UV C or VUV.
Similar effects of direct and indirect plasma treatment agree with the emission spectra, oxidation stress measurements and our previous findings [15]. Apparently, radicals and ROS (O, N, H, OH, O_3, O_2^-) represent the dominant biocidal agents in atmospheric air SC and TS discharges.

4. Conclusions

Bio-decontamination of water and surfaces contaminated by bacteria (*S. typhimurium* and *B. cereus*) was tested in two types of positive DC discharges in atmospheric pressure air in point-to-plane geometry. The streamer corona with small current pulses generates cold non-equilibrium plasma. With increasing applied voltage, the streamers transit to the novel regime transient spark with short (<100 ns) current pulses (~1-10 A) of 0.5–10 kHz repetitive frequency and very limited energy. Thanks to the very short spark pulse duration, the TS plasma remains relatively cold (~500 K).
Both SC and TS were found very efficient when the contaminated water was sprayed through the high voltage needle electrode and thus through the active discharge zone. EHDA effect occurring with corona discharge in this regime applied for the first time enhanced the efficiency of the process.
The comparisons of direct and two types of indirect exposure of contaminated agar plates to the plasma of SC and TS enabled the separation of the various biocidal agents. We demonstrated that the direct plasma and indirect exposure to separated active neutral species had almost the same effect on bacteria. On the other hand, separated plasma radiation, including UV, had no significant effect. These investigations, together with the emission spectra, indicated the major role of radicals and reactive oxygen species (O, OH, O_3). Their role in the plasma treatment was confirmed by the absorption spectroscopic detection of the products of cell membrane oxidation stress in TBARS method.
In summary, we demonstrated that cold atmospheric air DC discharges can be efficiently used for bio-decontamination of water and surfaces. The dominant biocidal plasma agents are radicals and ROS, which agrees with findings published by many other authors.

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