Streamer corona and transient spark in air for bio-decontamination of water and surfaces

Z. Machala[1], B. Tarabová[2], M. Pelach[2], K. Hensel[1], M. Janda[1], L. Šikurová[2]

[1]Division of Environmental Physics, [2]Division of Biomedical Physics,
Faculty of Mathematics, Physics and Informatics, Comenius University, 84248 Bratislava, Slovakia

Abstract: Bio-decontamination of water and agar surfaces contaminated by bacteria (*Salmonella typhimurium, Bacillus cereus*) was investigated in two types of positive DC-driven discharges in atmospheric pressure air, in needle-to-plane geometry: the streamer corona and the transient spark with short high current pulses of limited energy. Both discharges generate cold non-equilibrium plasma. Electro-spraying of the treated water through the needle electrode resulted in fast efficient bio-decontamination. Experiments comparing direct and indirect plasma effects, the emission spectra, oxidation stress measurements in the cell membranes, and chemical changes induced in the treated water helped better understanding of the plasma agents responsible for microbial inactivation. Radicals and reactive oxygen species seem to be dominant biocidal agents, although understanding of the plasma-induced water chemistry and the temporal evolution of the bio-decontamination mechanisms requires further research.

Keywords: bio-decontamination, air plasma, streamer corona, transient spark, electro-spray, oxidative stress

1. Introduction

Cold (nonequilibrium) plasmas at atmospheric pressure find recently numerous biological and bio-medical applications thanks to their reactive nature. In bio-decontamination by plasmas, it is crucial to understand the role of various mechanisms involved. In atmospheric pressure plasmas, the major role is typically attributed to radicals and reactive oxygen species (ROS, e.g. OH, O, O_3) and to charged particles, especially O_2^-, affecting the cell membranes. UV radiation plays a role only in UV C germicide region (220-280 nm) or in vacuum UV. In cold air discharges, UV C or VUV are usually not generated, so radicals and ROS are typically identified as dominant bio-inactivation agents.

In this paper, the biocidal effects of two atmospheric air plasma sources treating water and agar surfaces are investigated – positive DC *streamer corona* (SC) and *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 (electric field, charged particles, neutral active species, UV radiation) by coupling the electrical discharge characteristics, their emission spectra, and biocidal effects and measurements of the oxidative stress induced in microbial cells. Comparing direct and indirect plasma effects enables further separation of various biocidal plasma agents.

2. Materials and methods

The experimental set-up for DC discharges in point-to-plane geometry, with a high voltage hollow needle electrode enabling water flowing through the discharge zone was described previously in [1,2].

2.2 Flowing water treatment

The discharge set-up enabled the contaminated water to flow directly through the high voltage hollow 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 as shown in Figure 1.

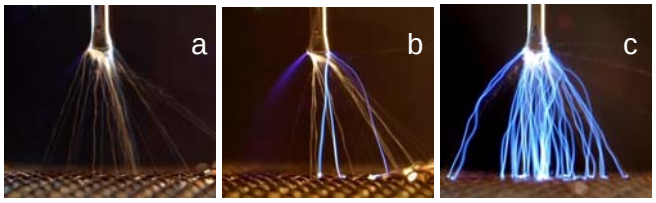


Figure 1. Photographs of the electro-spray of water in 8 mm gap, water flow rate 0.5 mL/min: (a) electro-spray with SC, 6.5 kV, (b) transition SC-TS, 7.8 kV, (c) spray with TS, 9 kV.

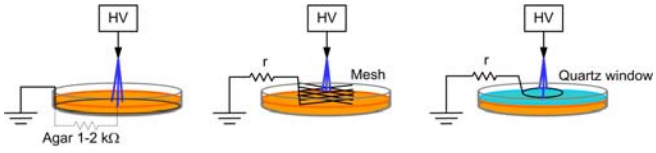
The experiments were repeated 5-10 times for each discharge type at various operation parameters.

2.3 Agar surface treatment – direct and indirect

We compared direct and indirect plasma effects on contaminated solid agar surfaces. A needle electrode was placed about 1 cm above the agar surface and the discharge was applied. In direct treatment, the agar was grounded with a wire. Indirect plasma effects were tested by placing the grounded mesh electrode ~2 mm above the agar, this shielded the electric field and expectantly trapped the charged particles, letting but neutral particles and partial UV light to reach the agar surface. We also tested an indirect exposure to UV (VUV) light from the discharge by placing a quartz (MgF_2) window onto the agar surface. [2]

Figure 2 depicts these arrangements. The agar in the direct treatment represents a certain electrical resistance, so a small resistor *r* was inserted in the indirect setup to simulate the agar's resistance.

These experiments were repeated in several series, 4-5 samples (dishes) for each specific set of parameters (exposure time, discharge parameters, etc.) were exposed in each series.



(a) (b) (c)

Figure 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 (or MgF_2) window transmitted only light from the discharge (UV).

2.4 Bacteria and their cultivation

Bio-decontamination effects of investigated DC discharges were tested on selected Gram-negative bacteria *Salmonella typhimurium* or Gram-positive *Bacillus cereus,* either suspended in deionised water, tap water and saline (physiological) solution with initial populations 10^6-10^7 colony forming units per mL (CFU/mL), or directly spread on the solid nutrient medium (agar, Roth Ltd.) on Petri dishes, about 10^5 per dish. Bacterial populations were determined by standard microbiological thermostatic (37 ℃) growth method with counting CFUs.

2.5 Measurements of the oxidative stress

Interaction of ROS with the bacterial cell membranes results in the peroxidation of membrane lipids, leading to malondialdehyde (MDA), quantifiable by spectrophotometry after the reaction with thiobarbituric acid (TBA) at 90–100 ◦C. This method of *thiobarbituric acid reactive substances* (TBARS) was applied to measure the oxidative stress induced in bacteria in water treated by SC and TS, similar to [1,2].

3. Results and discussion

3.1 Applied DC discharges

Two types of DC discharges of both polarities operating in atmospheric air with/without water were applied: *streamer corona* (SC) and *transient spark* (TS). Their electrical parameters and emission spectra were documented in detail in our previous works [3,4]. The streamer corona with small current pulses (~10 mA) and 10–30 kHz repetitive frequency generates cold (300–350 K) plasma. With increasing applied voltage, the streamers transit to the 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 (~500-1500 K, depending on repetitive frequency). The emission spectra and the measured temperatures indicate that both SC and TS generate non-equilibrium plasmas with various excited species (N_2^*), and radicals (OH, O, N, H).

3.2 Water treatment with electro-spraying

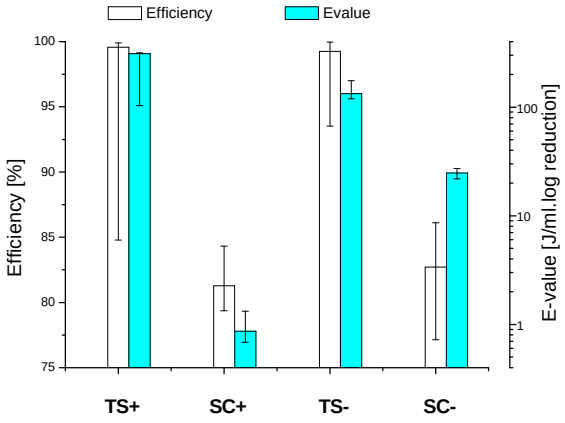


Figure 3. Comparison of inactivation efficiency of *B. cereus* in saline solution with *E-value*s for positive and negative TS and SC. Medians with 1st and 3rd quartiles as error bars. Typical number of repeated experimental sets was 10.

The water solution with bacteria was sprayed directly through the plasma active zone. As shown in Figure 3, the efficiency of transient spark was higher than of streamer corona. We use a new parameter *E-value* to express the combined energy requirements and efficiency of the process (Joule per treated water volume and one log reduction of microbial population). Streamer corona is far less energy-demanding than TS. 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 can be excluded.

3.3 Chemical changes induced in water

The chemical effects induced in the plasma treated water were measured by pH and conductivity probes, and spectrophotometric method for H_2O_2. Depending on the initial conductivity of the treated water (1, 500, 1000 μS/cm, physiologic solution ~14 mS/cm) and the plasma parameters, we observe a pH decrease from 5-7 down to 3-5 and an increase of conductivity. The measured peroxides (H_2O_2) reached up to 500 μM. pH decrease is probably due to the nitric acid formation. However, additional tests showed that the nitric acid solution of the same pH does not lead to the same biocidal effects. In agreement with [5], it is clear that acid environment in synergy with plasma agents leads to the bacterial inactivation.

3.4 Oxidative stress induced in bacteria

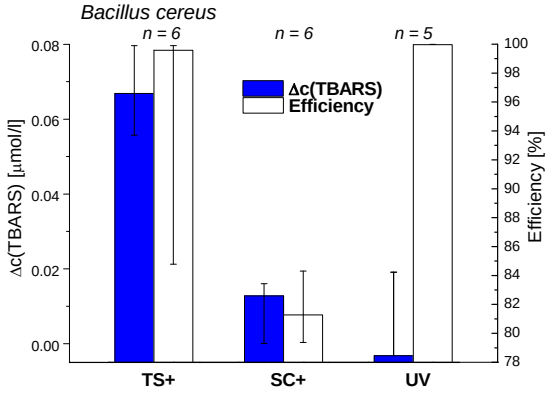


Figure 4. TBARS concentration gains and decontamination efficiencies of *B. cereus* in water treated by SC and TS with electro-spray, compared with 1 min exposure to UV C (Medians with the 1st and 3rd quartiles as error bars; n is the number of repeated experimental sets.

Figure 4 shows the inactivation efficiencies (*B. cereus*) for positive SC and TS with the electro-sprayed water and the measured concentrations gains *Δc*(TBARs). The same bacterial samples were irradiated by UV C radiation (Hg lamp, 254 nm, 1 min) for comparison. Concentrations Δc(TBARs) correlated with the inactivation efficiencies of the discharges. 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 especially TS treatments significantly enhanced the TBARs concentration. This indicates that oxidations of cell membranes by reactive oxygen species ROS are important in microbial inactivation.

3.5 Direct vs. indirect plasma treatment

A comparison of direct and indirect plasma effects on contaminated (*S. typhimurium)* agar surfaces was aimed at separation of various biocidal plasma agents. In indirect exposure, a grounded mesh filtered the charged particles and electric field from neutral radicals and excited species. Figure 5 shows the photographs of the contaminated agar surfaces after direct and indirect TS treatment. The effects of plasma on contaminated agar are visible as dark voids, whereas controls were homogeneously covered by cultivated bacteria.

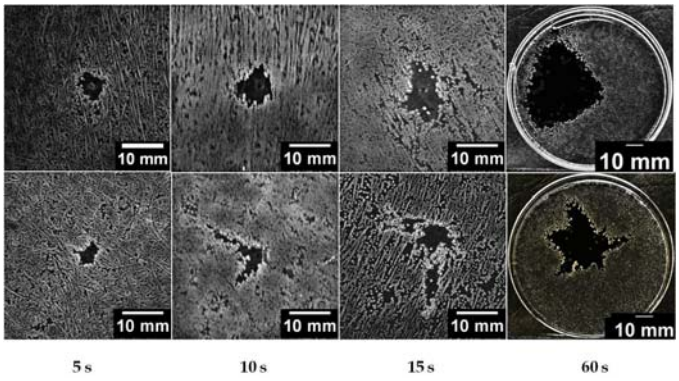


Figure 5. Photographs of contaminated agar surfaces with area inactivated by direct (upper row) and indirect (lower row) exposure to TS for various exposure times. 60 s (far right) is not in the same scale.

Both direct plasma and indirect exposures to neutral reactive species caused apparent bio-decontamination (voids). The areas of inactivated voids were measured by processing the Petri dish photographs in ImageJ software and statistically evaluated.

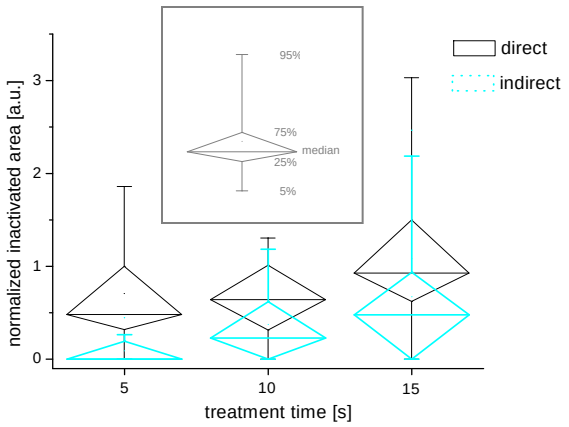


Figure 6. Normalized inactivated area as a function of treatment time for direct and indirect exposures to TS. Medians, 1st and 3rd quartiles, and 5th and 95th percentiles; statistics of 3 repeated experimental series, 2x3x5 samples each.

Figure 6 shows the inactivated area (normalized through 3 experimental series) for direct and indirect exposure for short treatment times (5-15 s). The inactivated area increases with the exposure time. The direct exposure is much stronger for 5 s, which indicates that charged particles are important at the beginning of the treatment. However, at 15 s the direct and indirect effects become more similar. At 60 s treatment, there is very little difference between the direct and indirect exposures, indicating a crucial role of reactive neutral species.

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 UV C or VUV light.

4. Conclusions

Bio-decontamination of water and surfaces contaminated by bacteria was tested in two DC discharges in atmospheric pressure air: streamer corona and transient spark. Both were found very efficient when the treated water was sprayed directly through the high voltage needle electrode and thus through the active discharge zone. The role of reactive oxygen species (O, OH, O_3) was confirmed by the absorption spectroscopic detection of the products of cell membrane oxidation stress.

The comparisons of direct and indirect exposure of contaminated agar surfaces to SC and TS enabled separation of various biocidal agents. Both direct plasma and indirect exposure to active neutral species only had almost the same effect on bacteria at exposure times from 60 s, whereas direct plasma effect was stronger at very short exposures (<15 s). The detailed mechanism has to be further studied, so far it seems that charged particles play a role in very short exposures and neutral species (such as ROS) are crucial at longer exposures. Separated plasma UV radiation had no significant effect.

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References

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