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Decontamination of Streptococci biofilms and Bacillus cereus spores on plastic surfaces with DC and pulsed corona discharges ⋆

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Abstract. Cold air plasmas of DC and pulsed corona discharges: positive streamers and negative Trichel pulses were used for bio-decontamination of *Streptococci* biofilm and *Bacillus cereus* spores on polypropylene plastic surfaces. The reduction of bacterial population (evaluated as $\log_{10}$) in the biofilm on plastic surfaces treated by DC corona reached 2.4 logs with 10 min treatment time and 3.3 logs with 2 min treatment time with water spraying. The enhancement of plasma biocidal effects on the biofilm by electro-spraying of water through a hollow needle high-voltage electrode was investigated. No significant polarity effect was found with DC corona. Pulsed corona was demonstrated slightly more bactericidal for spores, especially in the negative polarity where the bacterial population reduction reached up to 2.2 logs at 10 min exposure time.

1 Introduction

Non-equilibrium (cold) atmospheric-pressure plasmas possess unique characteristics enabling to use it for various applications, including quickly growing biomedical ones. Thanks to the low gas temperature, non-equilibrium plasma may not cause any thermal damage to the matter it comes in contact with.

Biological decontamination of thermo-sensitive materials by non-equilibrium plasma has been widely studied recently. The new plasma applications can be found, for example, in the provision of the food safety (disinfection of the food and food packages) [1], the tissue treatment [2], chronic wound healing [3], blood coagulation [4], catheters' sterilization [5,6] and in dentistry [7]. Besides biofilm decontamination and root canal disinfection [8,9] in dentistry, teeth whitening with the use of plasma has become a popular subject of study [10].

Various contamination agents can be efficiently treated by plasma, for example: fungi, yeasts, bacteria, etc. Even resistant bacterial species (e.g. MRSA), biofilms and bacterial spores, which are not easily decontaminated by conventional chemical agents, could be eliminated by plasma [11–16]. Plasma treatment has also promising applications in cancer therapy [17].

Plasma chemical effect on a bacterial cell membrane is highly dependent on the amount of water in the sample. The highest biocidal effect was found on moist samples but a large amount of water causes a dilution effect [18]. Electrostatic spraying [19,20] of water with a small flow rate on a sample during decontamination could possibly have the same effect as the sample moistening before the decontamination [11].

Recently, we have been working on decontamination of bacterial spores [11] and bacterial biofilm, which is another very resistant bacterial community. Human tissues support complex microbial communities growing as biofilms that can cause a variety of infections. As a result of an increased use of implanted medical devices and long-term catheters, the incidence of biofilm-associated diseases is increasing. Bacteria in biofilms exhibit increased tolerance toward commonly used antimicrobial agents and cold air plasma can be an alternative method for disinfection and sterilization of the thermo-sensitive materials supporting their growth. Plasma used for biofilm decontamination has been relatively little tested so far. It is capable of disinfecting dental plaque or caries and so may become an attractive painless alternative to teeth drilling.

In our work we tested the decontamination of oral *Streptococci* biofilms cultivated on and spores of *Bacillus cereus* spread on plastic surfaces. We investigated

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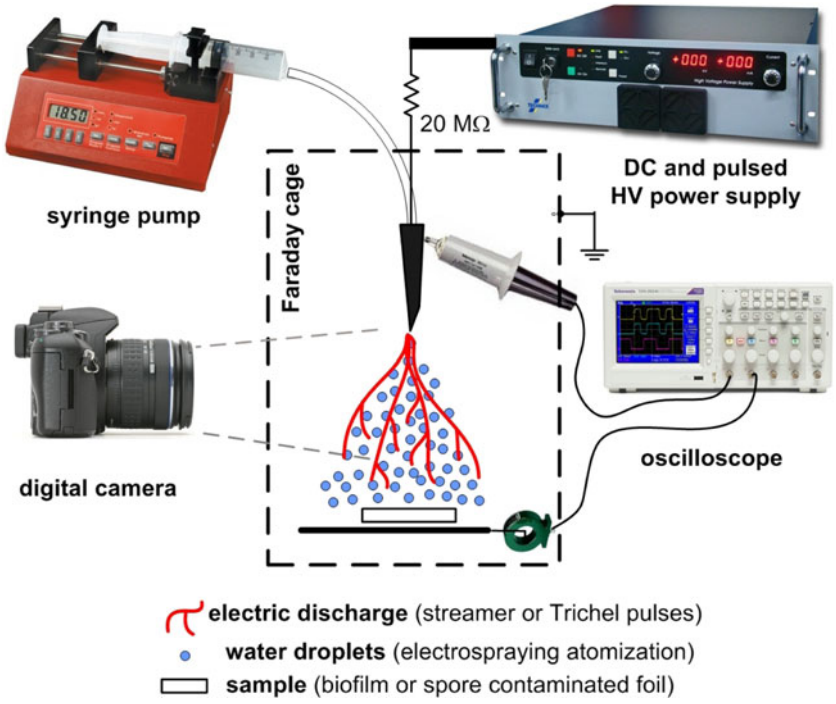

Fig. 1. Schematic of the experimental setup.

the influence of treatment time and energy and the effect of electrostatic water spraying through the discharge on the inactivation of spores and bacteria in biofilm.

2 Methodology

DC and pulsed corona discharges were used for decontamination of *Bacillus cereus* spores on plastic surfaces. DC corona discharge of both polarities was tested for biodecontamination of *Streptococci* biofilms (from normal oral flora) on plastic surfaces. An effect of electro-spraying on efficiency of decontamination was applied.

2.1 Experimental setup

The experimental setup consisted of a DC or pulsed high-voltage (HV) power supply, a discharge chamber placed in a Faraday cage and a digital oscilloscope Tektronix TDS 2024. The voltage was measured with a high-voltage probe Tektronix P6015A and the current on a 50 Ω grounded resistor connected through a coaxial cable to the oscilloscope. In some experiments, tap water was electro-sprayed on the samples. Water was pumped by an injection pump SiringePump NE-300 with adjustable flow rate, through the HV electrode (specially modified for electro-spraying) and the discharge zone. The photographs were taken by Olympus E410 camera (Fig. 1).

The discharge chamber contains a sharp hypodermic injection needle as a high-voltage electrode opposite to a grounded stainless-steel mesh or a copper plate. The treated polypropylene foil samples were placed on the grounded electrode. The distance between the HV electrode and the sample was 5 mm.

2.2 Discharges used

Positive streamer corona was supplied with a positive DC HV from 5 to 8 kV for plastic surfaces with biofilms and 10 kV for plastic surfaces with spores. On both types of samples, the corona forms streamers (current pulses) with frequency 10–20 kHz and maximum amplitude up to 150 mA for the smallest frequencies (Fig. 2). The amplitude of current pulses decreased with increasing voltage.

Negative Trichel pulses were supplied with a negative DC HV from 4 to 8 kV for the samples with biofilm and ∼9 kV for the samples with spores. We detected <0.7 mA current pulses with ∼500 kHz frequency (Fig. 3) on biofilm samples and <0.35 mA pulses with ∼1 MHz on samples with spores. With water spraying, the formed current pulses were up to 9 mA amplitude with frequency 60 kHz.

Pulsed corona. Besides using DC power for the corona discharge generation, we have alternatively used pulsed power in a way to evaluate and compare the biocidal effects of the two power supplies. A rotating spark gap power supply was used to generate the pulsed corona discharge of positive and negative polarities. The pulse-forming circuit consisted of HV transformer (800 W), storage capacitor (9 nF) and rotating spark gap chamber capable of providing pulses of repetition frequency up

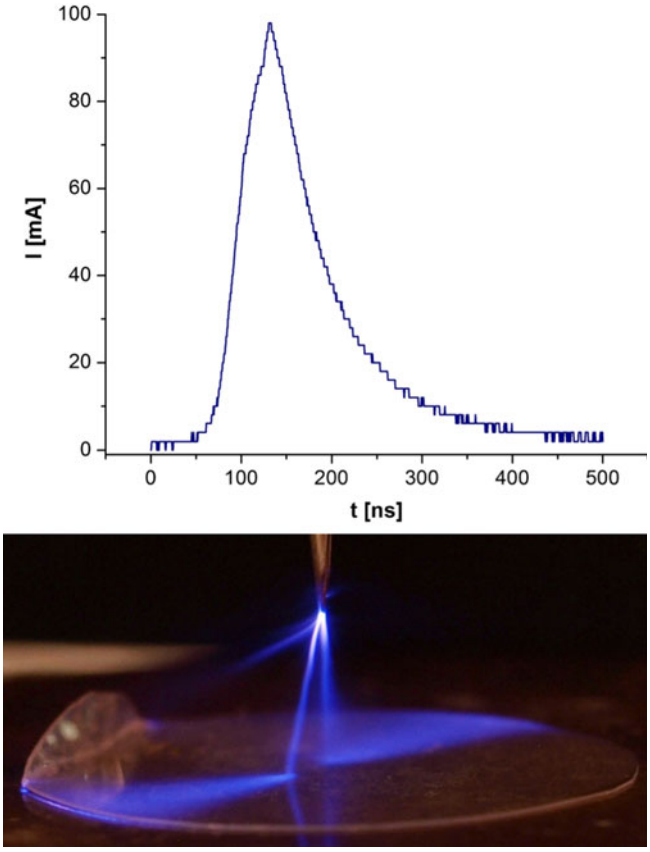

Fig. 2. The oscilloscopic record of positive streamer current pulse ($U = 6$ kV, $f = 15$ kHz, Imax $= 100$ mA) and the related discharge photograph.

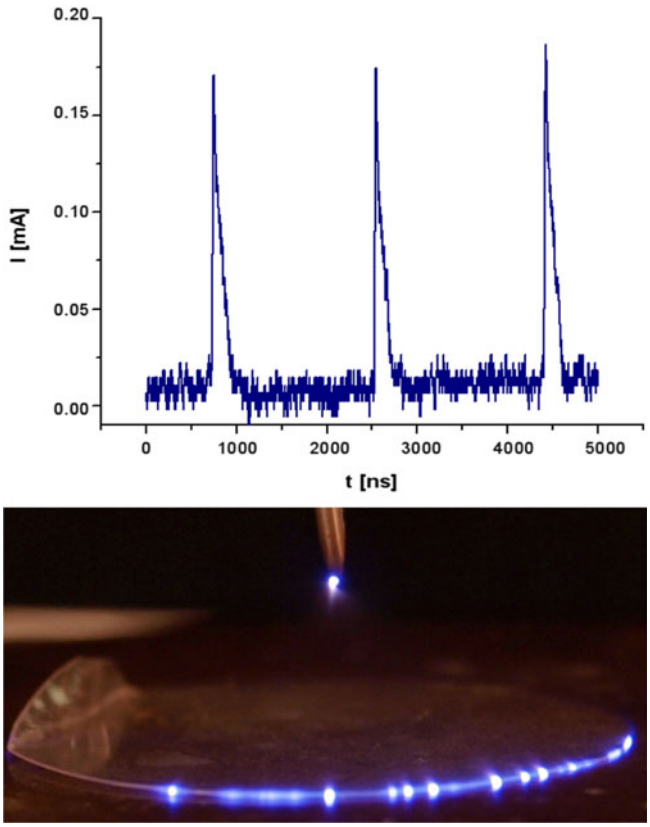

Fig. 3. The oscilloscopic record of the current of negative Trichel pulses ($U = 7$ kV, $f = 550$ kHz, Imax $= 0.2$ mA) and the related discharge photo.

to 266 Hz. The typical waveform of the applied voltage signal is presented in Figure 4. The polarity of the power supply was switched by reversing the position of a rectifier. The rotating spark gap power supply represents a simple and a cheap pulsed power supply that has been efficiently used for various environmental applications in air and water [21,22]. In the present work, the pulsed power was used only for the treatment of spores deposited on the plastic surfaces without water spraying.

2.3 Experimental procedure

2.3.1 Sample preparation

The polypropylene foil samples (round shape with 2 cm diameter) were sterilized under UV-C lamp ($\lambda = 254$ nm, $P = 11.2$ W) from both sides prior to application of biofilm or spores.

The biofilm was produced by Viridans group of genus *Streptococcus* taken from oral microflora of three volunteers (22-year-old woman and two 23-year-old men). The culture taken from the volunteers was cultivated on *Streptococci* selective agar (Biolife). The overnight culture was produced from selected colonies. On the next day, we cultivated 24-h biofilm on plastic surfaces in a special receptacle. The bacterial suspension was diluted in fresh soyabean casein digest medium (Biomark laboratories) enriched with 4% glucose solution (the number of bacterial cells was 10^8 cells per mL). After 24 h of biofilm cultivation on the plastic surfaces, these samples were rinsed in a sterile physiological saline solution to remove unattached bacteria.

The bacterial spores of *Bacillus cereus* were prepared from suspension of germinative bacteria. A small volume (50 μL) of *Bacillus cereus* spore suspension was spread over the plastic and dried at 35 °C overnight.

2.3.2 Discharge treatment

In the experiments *without water spraying*, the plastic foils were put on a sterile copper plate grounded electrode and treated in the discharge for 2, 5 and 10 min (biofilm) or 1, 2 and 5 min (spores).

In the experiments *with water spraying*, the plastic foils with biofilm were put on a stainless-steel mesh grounded electrode and treated in the discharge for 2 min. The tap water was sprayed through the HV electrode (a hollow injection needle) with the flow rates 0.01 and 0.05 mL/min.

During the sample decontamination, we recorded electrical discharge characteristics to calculate the discharge power and evaluate energy consumption. After the treatment, the plastic foil was put in the test tube with a sterile

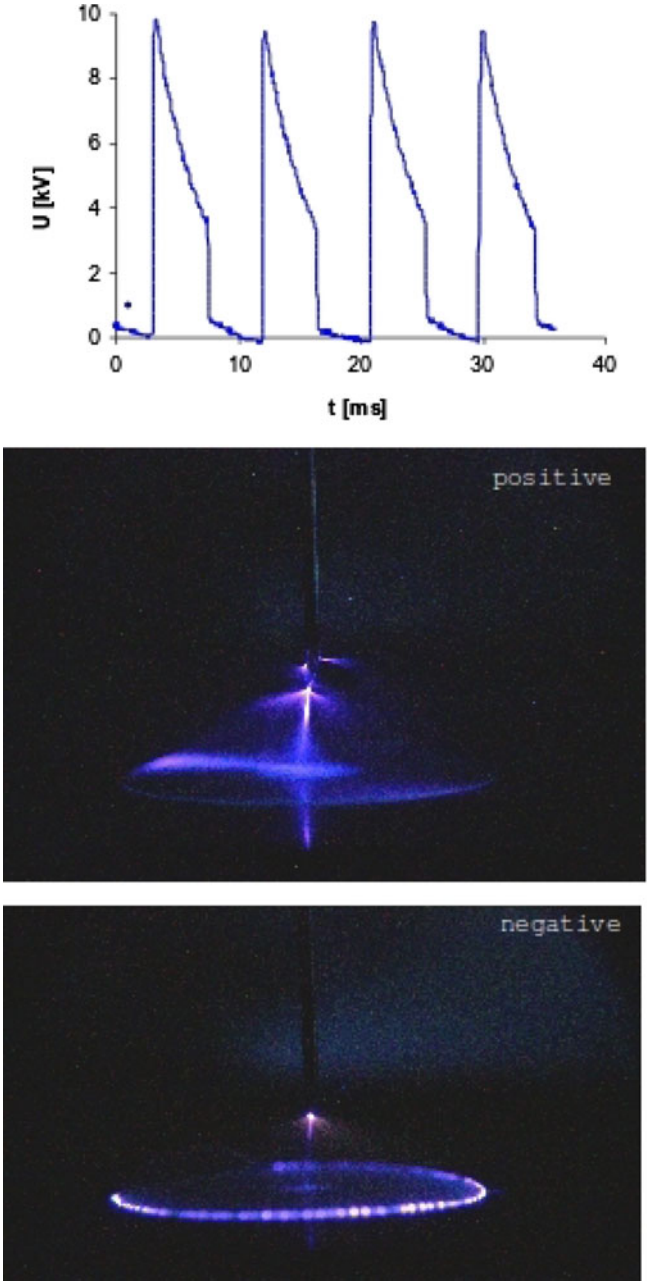

Fig. 4. The oscilloscopic record of applied pulsed HV voltage waveform (U = 10 kV, f = 100 Hz) and the corresponding images of the discharges generated in positive and negative polarity above plastic surfaces contaminated with spores.

physiological saline solution. This procedure was repeated for all samples.

2.3.3 Sample cultivation and result evaluation

The samples in test tubes were ultrasonicated for 20 min to detach biofilm and spores from foil surface. We used Vortex classic for homogenization of the samples. Each sample diluted in the saline solution was spread on agar in three Petri dishes. Reference samples were prepared in the same way without exposure to the plasma. The Petri dishes with inoculated bacteria were cultivated overnight at 35 °C.

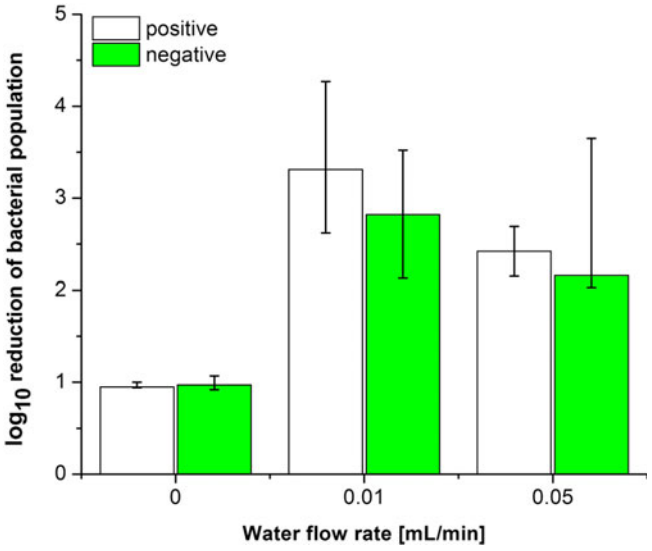

Fig. 5. Reduction of bacterial population of *Streptococci* biofilm on plastic surfaces by corona discharges with water electro-spraying for different flow rates (exp. time = 2 min) (medians with IQR).

Efficiency of discharge decontamination was determined as a decadic logarithm from the number of bacteria in the reference sample divided by the number of bacteria in the treated sample.

We used median and interquartile range (IQR) for representation of data sets because of non-normality of the data. All statistical tests were made at the level of significance 5%. The comparison of positive and negative DC and pulsed corona $\log_{10}$ reduction in three flow rate categories and four exposure times was done by non-parametric Mann-Whitney U test for medians. Pairwise comparison between three flow rates was done by non-parametric Kruskal-Wallis test for analysis of variance.

3 Results

3.1 Decontamination of biofilm samples

3.1.1 Without water spraying

The experiments with *Streptococci* biofilms were performed in five series with average number of contaminated biofilm samples being 10. The three exposure times were used: 2, 5 and 10 min.

Figure 5 shows $\log_{10}$ reduction results on plastic surfaces. Reduction of bacterial population increased with the exposure time reaching up to 2.5 logs at 10 min. Differences in $\log_{10}$ reductions between positive and negative DC corona discharges were not significant. Corresponding energy consumption was comparable for both polarities of corona and it increased insignificantly with the exposure time. Medians of energy varied from 10.4 J to 33.1 J.

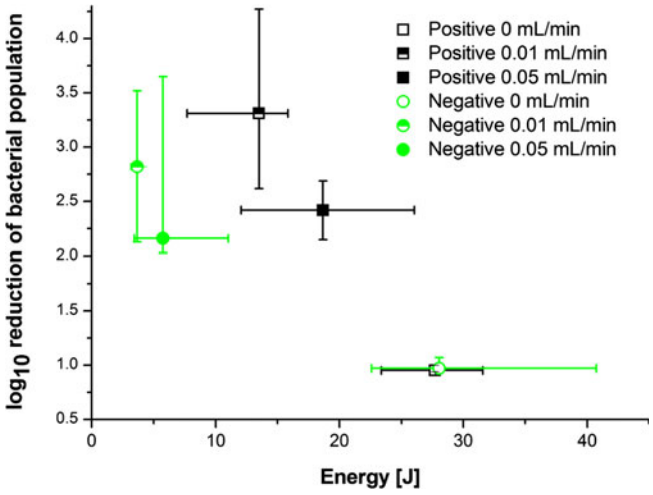

Fig. 6. Reduction of bacterial population as a function of energy consumption. Plastic surfaces contaminated by *Streptococci* biofilm, exposed to DC corona discharges with water electro-spraying for 2-min exposure time (medians with IQR).

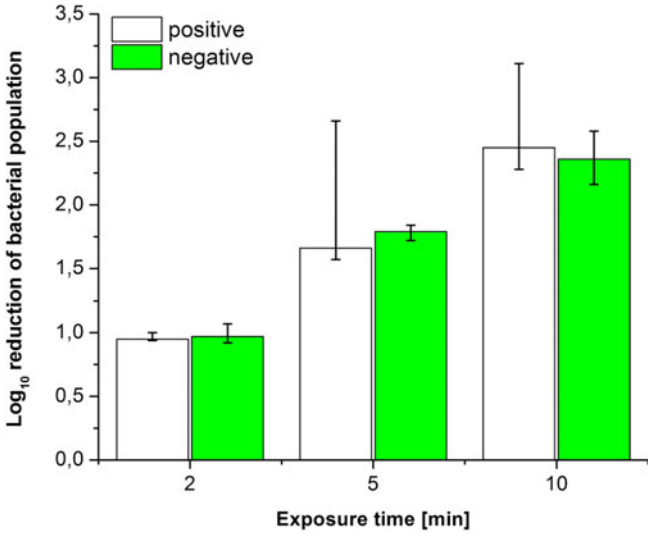

Fig. 7. Reduction of bacterial population on plastic surfaces contaminated by *Streptococci* biofilm by DC corona discharges for three different exposure times (medians with IQR).

3.1.2 With water spraying

Figure 6 shows $\log_{10}$ reduction of bacterial population in DC corona discharges with water spraying for two flow rates compared with no spray (flow rate 0 mL/min) at exposure time 2 min. Differences in $\log_{10}$ reduction between the positive and the negative corona discharges were not significant at all flow rates tested. The electro-spraying significantly increased $\log_{10}$ reduction from 0.95 logs up to 3.3 logs ($p < 0.0001$). For the positive corona, the difference between flow rate 0.05 and 0.01 mL/min is marginally significant ($p = 0.0531$), with 0.01 mL/min more efficient. For the negative corona, there is no significant distinction between the two flow rates.

As shown in Figure 7, the electro-spraying of water on the biofilm samples during decontamination significantly reduced the energy consumption. In all cases with water spraying the negative corona consumed less energy than positive.

3.2 Decontamination of spores

3.2.1 DC and pulsed corona discharges

Experiments with *Bacillus cereus* spores treated by DC corona were performed in 3 series with 10 contaminated plastic foils in each. The experiments with pulsed corona were performed in 3 series with 3–5 contaminated plastic foils. Four exposure times were used: 1, 2, 5 and 10 min for pulsed corona and 2 and 5 min for DC corona. Figure 8 summarizes the results of the reduction of the bacterial population as a function of treatment time for both DC and pulsed polarities. The $\log_{10}$ reduction mostly increased with the treatment time. The maximum $\log_{10}$ 2.2 was found after 10 min with negative pulsed discharge.

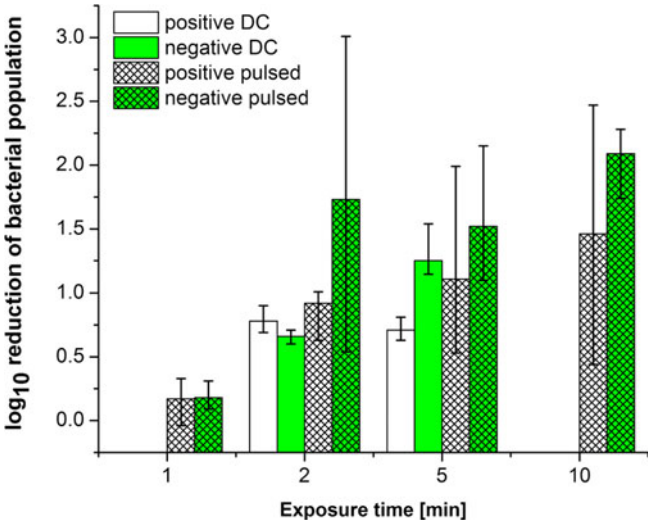

Fig. 8. Reduction of bacterial population of *Bacillus cereus* on plastic surfaces by DC and pulsed corona for four different exposure times (medians with IQR).

4 Discussion

Experiments with biofilm decontamination by DC corona demonstrated significant enhancement of the bactericidal effect due to electro-spraying of water through the discharge. The main cause of this effect is still under scientific investigation. In ambient air plasma, reactive oxygen species (e.g., atomic oxygen, metastable oxygen, ozone and OH radicals) are generally accepted to play dominant roles in cellular inactivation. In the plasmas in air-water environments, the presence of water adds more complexity to the system and forms reactive oxygen and nitrogen species. Based on our experiments focused at chemical effects in water sprayed through air transient spark discharge [23] and in agreement with other recent

works [24–26], we can hypothesize that the strong enhancement of biofilm inactivation can be attributed to nitrites, hydrogen peroxide and peroxynitrates formed in the electro-sprayed water.

The slight decrease in $\log_{10}$ reduction with water flow rate 0.05 mL/min as opposed to 0.01 mL/min was probably caused either by not so efficient electro-spraying, or by forming a thin protective water layer on the surface of biofilm preventing further plasma-activated water from affecting the surface.

The electro-spraying of water also resulted in the decrease of energy consumption. This energy reduction was due to lowering the pulse repetition frequency and so the power.

The effect of copper ions on the bacteria viability from grounded electrode could not be observed. In the case of plastic foil surfaces without water spraying, bacteria were only on the upper side of the plastic foil, which was not in contact with copper plate. When using electrostatic spraying some diffusion of ions from the ground electrode onto the treated surface could be possible through a thin humid layer, the stainless-steel mesh was used as the grounded electrode, not copper.

Experiments with *Bacillus cereus* spores comparing the effects of DC a pulsed corona discharges (without water spraying) demonstrated slightly higher log reductions with pulsed corona. In general, negative polarity was found to be more efficient than positive. However, a direct comparison of the data obtained with the two power supplies is so far impossible, as the energy consumption must be taken into account, which requires further work planned in the near future.

5 Conclusions

We tested the bactericidal ability of cold plasma of corona discharges: DC positive streamer corona and negative Trichel pulses and positive and negative pulsed corona on plastic surfaces contaminated with bacterial *Streptococci* biofilm and *Bacillus cereus* spores. The DC corona discharge reduced bacterial population in biofilm by up to 2.4 logs in exposure time 10 min. Electro-spraying of water through the plasma significantly enhanced this effect. In 2-min exposure time, decontamination effect increased from 0.95 logs up to 3.3 logs reduction. For biofilm contamination, DC positive corona with the flow rate 0.01 mL/min was the most bactericidal.

The bactericidal activity of DC and pulsed corona discharges on the plastic foils contaminated by *Bacillus cereus* spores was found slightly weaker than on biofilms without water spraying. Pulsed corona was demonstrated slightly more bactericidal, especially in the negative polarity.

In summary, we found that both DC and pulsed corona discharges of both polarities are able to effectively reduce bacterial population of the biofilm and spores on plastic surfaces and so can be used as an alternative to chemical and mechanical bio-decontamination methods, which are currently used in medicine. Water electrospraying can be employed as an efficient enhancement of the bactericidal effect.

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