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DC corona discharges in air for bio-decontamination of glass surface from Escherichia coli

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Abstract: Corona discharges in air in positive and negative polarity were used for bio-decontamination of glass surfaces from planktonic *Escherichia coli* and its biofilm. In order to enhance the bactericidal effect of the plasma, water was electrosprayed from a hollow electrode onto the sample for either half or the entire treatment time. The best bio-decontamination efficiency of 4.5 log was achieved with electrospray during 20 min of treatment by positive streamer corona. Our first results on *E. coli* biofilm treatment are presented and a possible loss of biofilm biomass is measured. In order to better understand the processes of bacteria inactivation, the electrosprayed water was chemically analysed.

Keywords: *Escherichia coli*, Bacterial biofilm, Corona discharge, Bio-decontamination, Electrospray

1. Introduction

Bacterial contamination of surfaces is a common problem for patients in hospitals, for the food industry, water distribution systems, etc. In order to avoid using toxic chemicals to achieve desired decontamination efficiency, we need to seek for new alternative methods of decontamination. Non-thermal plasma at atmospheric pressure is well-adapted for decontamination of thermally sensitive surfaces because it produces radiation (UV, visible, IR), electromagnetic fields, excited species, radicals and various chemical products, free electrons and ions, while bulk temperature remains close to ambient. Its bactericidal effect has been previously tested on a range of bacterial species - planktonic bacteria, spores or bacterial biofilms. Electrosprayed water brings more complexity to the discharge chemistry and its interaction with bacteria. The effect of water electrospray combined with corona discharges has been previously studied in our group [1, 2] and applied to the biofilm and spore decontamination of plastic surfaces [3, 4] and to water disinfection [5, 6]. In this paper we investigate the impact of water electrospray and the effect of the convective transport of neutral species produced in the plasma from the surface of planktonic *Escherichia coli*.

2. Materials and methods

Both positive corona (PC) and negative corona (NC) discharges in air were studied for bio-decontamination of *Escherichia coli* in both planktonic and biofilm form on glass surfaces. We tested the effect of water electrospray through the discharge on its bactericidal efficiency.

2.1 Experimental set-up and discharges

Corona discharges at atmospheric pressure in air were generated in an experimental set-up consisting of a DC high-voltage (HV) power supply and a discharge chamber. The discharge chamber contained a sharp or a clipped hypodermic syringe needle as HV electrode opposite a grounded copper plate. Treated samples were placed on the grounded electrode, 5 mm from HV electrode. Some experiments were done with sterile distilled water electrospayed onto the sample, by pumping (with SyringePump NE-300) through a hollow clipped HV electrode. Electrical characteristics of the discharges were measured: the voltage with a Tektronix P6015A HV probe and the electrical current on a 50 Ω grounded resistor connected through a coaxial cable to a Tektronix TDS 2024 digital oscilloscope.

Corona discharges of both polarities were used in point to plane configuration. Positive streamer corona (PC) was supplied with a voltage up to 9 kV and formed streamers with frequencies from 10 - 20 kHz and maximum amplitudes up to 100 mA with or without water spraying. NC was supplied with a maximum voltage of 8 kV and current pulses with frequencies from 0.5 to 2 MHz and amplitudes up to 1 mA were observed with or without water spraying. More details on the discharge experimental conditions can be found in [4].

2.2 Bacterial samples

In all experiments we used glass cover slides (2×2 cm) as substrates for the bacterial samples. Three different *Escherichia coli* strains were used for this study: DH1, BW25113 and BW25113 with plasmid F (F+).

In the case of planktonic bacteria, a frozen aliquot (DH1) in 20 % glycerol was solubilized in 1/3 Miller's modified Luria broth (LB) medium and 10 µL of suspension was dripped in the middle of a sterile cover glass and immediately treated by the plasma. Ten µL of *E. coli* BW25113 overnight culture was also placed in the

middle of the cover glass and dried for 20 min at 37°C. For both cases the final concentration on the cover glass was 10^6 bacteria/mL. After plasma treatment, bacterial cells were recovered with 10 repetitive rinsings of the surface with a total of 100 µL distilled water and immediately resuspended in 1/3 LB. The samples were then serially diluted and spread over the agar surface in Petri dishes and incubated for 24 hours at 37°C; the resulting colonies were then counted.

E. coli BW 25133 F+ was used to form biofilm on glass slides for 48 hours at 30°C. An overnight culture was diluted in supplemented M63 media and 1 mL of this suspension was pipetted into each well of home-made 6-well plate with cover glasses on the bottoms. After 24 hours media were replaced by fresh one and the biofilm was treated after 48 hours of incubation and drying. The biofilms after treatment were either scraped into buffer solution, diluted and plated on agar for colony counting or used to measure its biomass by crystal violet (CV) colorimetric assay [7]. In this assay, the biomass of the biofilm is stained by 0.1 % water solution of CV, incubated, and excess CV is then washed out. After drying, CV is solubilized by 30% acetic acid and the absorbance at 550 nm is measured.

3. Results

The results from three planktonic experiments and preliminary results from measurements with biofilm are now to be presented.

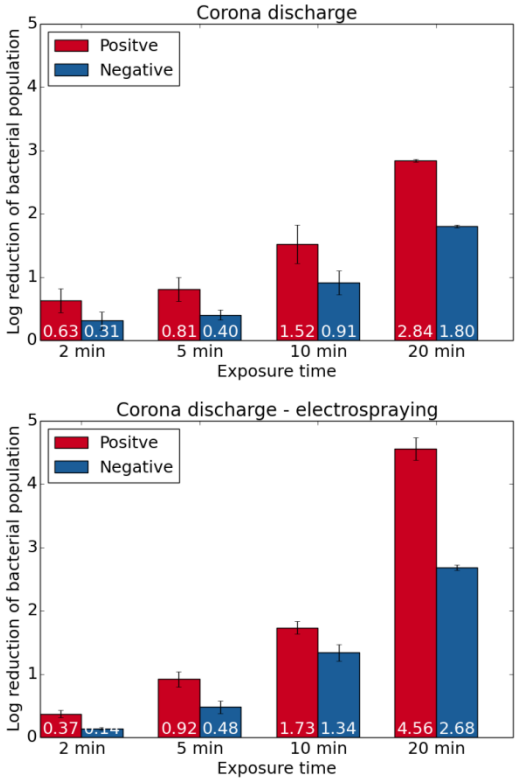

Fig. 1. Logarithmic reduction of bacterial population for four different exposure times to the corona discharges (a) in ambient air (b) with electrospraying of water through the discharge (3-7 replicates, mean±SEM).

3.1. Decontamination of planktonic E. coli DH1

First, we performed experiments with *E. coli* DH1 to compare PC and NC bactericidal efficiency with or without electrospraying of water. These results are presented in Figure 1.

PC reduced bacterial population within 20 min by almost 3 logs, with the addition of water electrospray by 4.6 logs. NC was less efficient: 20 min exposure caused 1.8 log and with water electrospray 2.7 log reduction. Even from the beginning of decontamination PC efficiency was always higher, this is because the measured input power for PC was 100 times higher than for NC (≈40 mW as opposed to ≈300 mW). Due to the fact that for experiments with water electrospray we used clipped hollow needle we created two sharp points from which coronas were originated (Figure 2). By creating two sharp points, the corona discharges were able to treat larger areas on a sample. This could also contribute to overall better efficiency of discharges with electrospray.

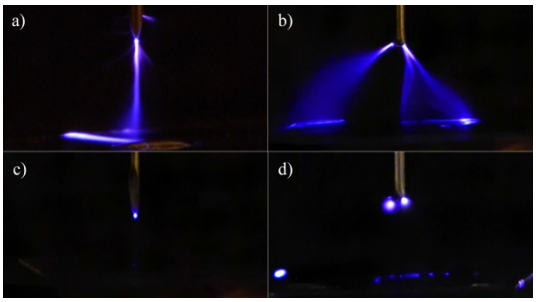

Fig. 2. Photos of air corona discharges applied on glass cover slides, first row is PC a) without electrospray with sharp needle, b) electrospray with clipped needle. Second row is NC c) sharp needle, d) clipped needle with electrospray.

3.2. Decontamination of planktonic E. coli BW25113

In these experiments we compared decontamination efficiency of electrospraying for PC and NC. In previous experiments with longer exposure times and the electrospray we noticed the formation of a large drop of water on the cover slide, this drop progressively changed the geometry of discharge chamber and caused occasional sparking. Therefore, in order to avoid the large water drop formation and to shorten the exposure time, we did all experiments for 5 min exposure time, including a 2.5 min corona treatment with electrospray and the rest of the time with corona discharge only. Another difference from the previous experiments is the *E. coli* BW25113 strain, which was chosen after failed attempts to form thick-enough biofilms from DH1. The results are presented in Figure 3.

The corona discharges only lead to 2.33 log reduction in the case of PC and 3.20 for NC. This bactericidal effect was significantly elevated by 0.6 log by the electrospray

of water through the discharges in both cases. Another 0.3 log increase was achieved by condition where the electrospray was used for half of the exposure time only. In contrast with the previously described experiment, NC was more efficient in reduction of bacterial population than PC. It is likely because we increased the power of NC (240 mW) to the same magnitude of PC (230 mW).

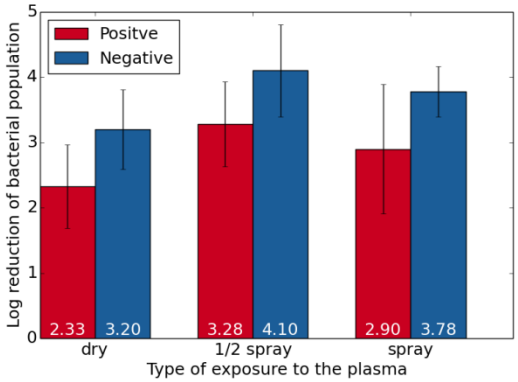

Fig. 3. Logarithmic reduction of bacterial population for three different exposure conditions to corona discharges (3-5 replicates, mean±SD).

3.3. Effect of separated charged particles from corona discharges on E. coli

In this experiment, the reaction chamber was continuously blown by the external ambient air inflowing through a tube close to the HV electrode, the flow rate of air was 4 slm, in the concept similar to [8]. By this configuration, we suppose to blow away neutral reactive species and therefore the bacterial samples with dried *E.coli* BW25113 were exposed to only charged particles (ions) from the discharge and to UV emission. From our previous measurements with the streamer corona and a transient spark [5], we had found that UV emission from these two discharges had no significant effect on bacterial survival. Comparison of the effect of the full plasma exposure with only charged particles formed in plasma is presented in figure 4.

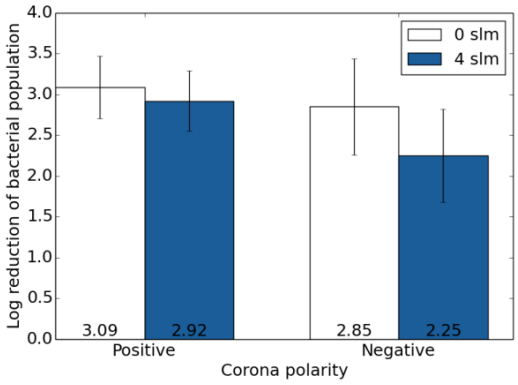

Fig. 4. Logarithmic reduction of bacterial population by the corona discharges with or without blowing of air through the discharge, exposure time 5 min (5-8 repeats, mean±SD).

Within 5 min of decontamination without air blowing we reduced the bacterial population by 3.1 and 2.85 logs by PC and NC, respectively. With blowing air (4 slm) through the discharge region we obtained slightly lower bio-decontamination efficiency, this difference was significant only for NC. This unexpectedly small reduction in efficiency can be caused by insufficient elimination of neutral species and can be improved by increasing air flow rate. However, increase in air flow rate affected the discharge electrical properties and caused the same and even higher efficiency of the discharge decontamination, so these results (not shown) cannot be directly compared to separate the effects of neutral and charged particles.

3.4. Preliminary biofilm results

The biofilms of *E. coli* BW25113 F+ growing for two days were treated by PC and NC for 5 or 10 min, without electrospraying of water. In these conditions within 10 min we obtained reduction of bacterial population by 2.8 and 2.64 logs in PC and NC treatment, respectively. CV assay was employed to test whether a biomass of the biofilm was etched during the plasma. Preliminary results do not show significant difference between absorbance at 550 nm of controls and samples treated by PC or NC for 5 and 10 min exposure times, indicating that the inactivation of the biofilm is not due to its etching.

4. Discussion

For the first two experiments with planktonic bacteria *E. coli* DH1 and BW25113 strains were used, the latter being considered as a wild type, thus less susceptible to outside stresses. From the results presented here, we can see that PC within 5 min of treatment causes a 0.81 log reduction for DH1 and 2.33 logs for BW25113. This can be the result of two factors, a high voltage electrode and preparation of a sample. Unlike DH1, BW25113 samples were dried before treatment and the HV electrode was clipped also for “dry” condition treatments. As we have already mentioned, by clipping the electrode, two sharp points are made and the discharge is able to cover a larger area on the sample. In addition, drying the sample prior to treatment can help ROS/RNS to affect the bacterial cells more directly.

Our preliminary measurements (methods described in [6]) of plasma induced chemistry in the electrosprayed water by PC and NC showed that multiple bactericidal agents were produced [4]. Namely H_2O_2, O_3 (both produced in bactericidal range), nitrites NO_2^-, and nitrates NO_3^-. The pH of the electrosprayed water decreased from 5.5 to 4 (NC) and 3.6 (PC), thus generating acidic conditions that influenced the chemistry. At lowered pH, nitrites are quickly oxidized to nitrates [6], and by reaction with H_2O_2 to peroxynitrites, which are associated with a strong bactericidal effect in plasma activated water [6, 9, 10], besides so-called acidified nitrite (i.e. nitrous acid), which is also associated with a strong bactericidal effect [9, 11]. Despite the fact that

these chemicals can act as bactericidal agents by themselves, the final bio-decontamination effect is reached by their combined effects [6]. The measurements with the positive streamer corona and the transient spark [5], showed that UV emission from these two discharges has no significant effect on bacterial survival. By blowing away the neutral species we were able to partially separate the effect of charged particles on bacteria. The slight decrease in bactericidal efficiency suggests that there is a significant contribution (in NC) of the charged particles on decontamination; we can speculate that these are dominated by superoxide anions O_2^-, since negative ions are abundantly formed in the drift region of negative corona in air. In synergy with neutral species, their effect is greater which agrees with the results of [8, 12].

5. Conclusion

Antibacterial effects of the positive streamer corona and the negative Trichel pulse corona were tested on *Escherichia coli* planktonic bacteria and in biofilms on cover glasses. For planktonic *E. coli* we obtained reduction of population by 1.8 - 2.8 logs within 20 min for DH1. This bactericidal effect was enhanced by electrospraying of water through the discharge and caused at the same time reduction of 4.5 logs in positive corona. For *E. coli* BW25113 there was also a rise of bactericidal effect with electrospraying of water, although it was higher for the condition when water was sprayed only for half of the treatment time. Corona discharge in air was able to successfully decontaminate 2.8 logs of bacterial population in biofilm. We found that low temperature plasmas of both polarities of DC corona discharges are able to reduce the bacterial population in *Escherichia coli* biofilm, and therefore are suitable for further study as alternative to conventional methods in decontamination.

6. Acknowledgments

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7. References

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