Dependence of bacteria and yeast inactivation on charge transported to the contaminated surface.

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The paper presents the dependence of exposed zone area and *Candida albicans, Escherichia coli* and *Staphylococcus epidermidis* inactivation on the charge, transported to a contaminated surface. A sharped pin electrode and the rotating agar surface as the second electrode was used to generate an atmospheric pressure negative corona discharge in ambient air. The high dependence of decontaminated area on the charge transported to the agar surface was shown for both gram-negative and gram-positive bacteria.

1. Introduction

Conventional methods of surface decontamination are based on chemical agents or heat application. These methods can be ineligible for heat-labile materials, food application etc. The plasma based decontamination seems to be a good alternative.

Non-thermal non-equilibrium plasmas, generated by different kind of discharges, produce three main bioactive agents. These agents are charged and neutral particles and UV light [1, 2, 3]. The role of each agent has not been investigated properly yet and remains the subject of discussion. The study presented in this article deals with the charge particles like the decontamination agent.

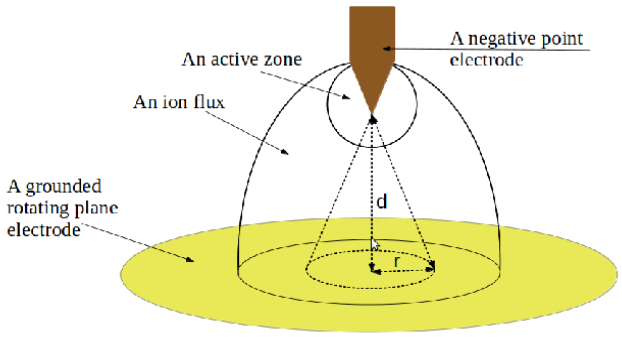


Figure 1. The ion and electron distribution along the gap between the electrodes. The dashed lines correspond to the Warburg's law description of radial current density distribution.

The Warburg's experiments [4] showed, that the plane electrode current density $j(\theta)$ for the point-to-plane corona discharge in air follows the law

$$j(\theta) = j(0)\cos^m(\theta), \quad (1)$$

where $j(0)$ is the current density in the electrode system axis position, i.e. right below the point electrode, θ is an angle coordinate, $\tan\theta = \frac{r}{d}, \theta \leq 60°$ (Figure 1). The parameter m was set to 4.65 as the best representation of experimental results according to Warburg's law.

$$j(0) = k\frac{U(U-V)}{d^n}, \quad (2)$$

where k is a constant, V is a corona breakdown voltage, U stands for discharge voltage, d is distance between electrodes. The parameter n was set to 3 according to the corona model published in [5].

2. Experiment

2.1. Apparatus

Low temperature plasma was generated by a DC negative corona discharge in ambient air. The point-to-plane electrode system configuration with a copper pin electrode was used. The plane electrode was realised by an ion conducting agar medium in Petri dish connected to an electric circuit with a copper strip. The plane electrode was rotating with angular frequency of 12π per minute. The voltage was set to 10 kV and applied current (100 μA) was set by adjusting the gap between electrodes (7 – 9 mm).

2.2. Samples

Candida albicans, *Escherichia coli* and *Staphylococcus epidermidis* were used as model organisms to investigate the dependence of decontamination on the amount of charge. All strains were obtained from Czech Collection of Microorganisms (CCM). These organisms represent yeast as a eukaryotic microorganism, gram-negative and gram-positive bacteria respectively.

The main difference of gram-positive and gram-negative bacteria is that gram-positive bacteria have a thick layer of peptidoglycan, which absorbs the gram stain. Gram-negative bacteria have a thick lipid bilayer on the outside, which is selectively permeable. The gram-positive bacteria are much more susceptible to antibiotics than gram-negative bacteria [6].

1 ml of inoculum suspension in saline was split onto a Sabouraud (for *C. albicans*) or Mueller Hinton (for *E. coli* and *S. epidermidis*) agar surface. It had been completely dried out in the flow box and then exposed to the plasma. Resulting concentration of yeasts or bacteria on the agar surface was 10^7 cfu/ml.

2.3. Exposition

Table 1. Radial displacement of the pin electrode and exposition time of different experiments. Codes 0+2 etc. stand for subsequent exposition of the same agar.

Radial displacement of a pin electrode [cm]	Exposition time [min]		
0	3	-	-
1	3	-	-
2	3	6	-
3	3	6	9
0 + 2	3 + 3	3 + 6	-
0 + 3	3 + 3	3 + 6	3 + 9

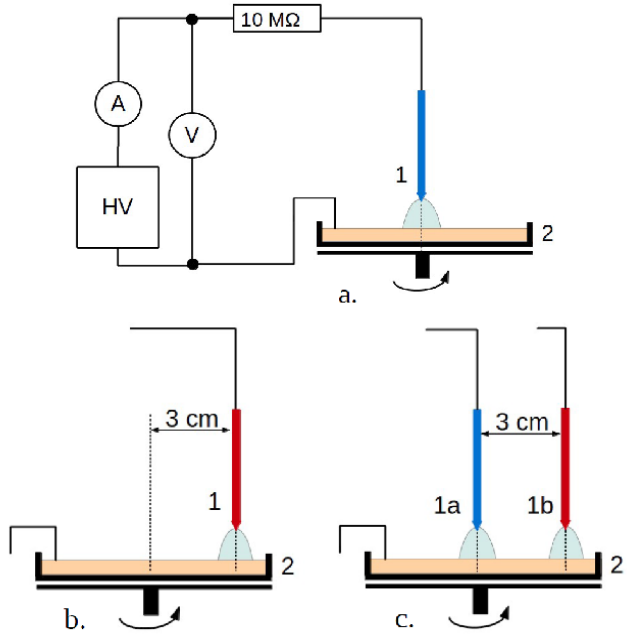


Figure 2. The point (1) to plane (2) electrode configuration with rotating plane electrode; a. the reference measurement with no radial displacement; b. the radial displacement, c. subsequent exposition of the same agar.

Presuming, that decontamination depends on charge, several experiments were performed. The amount of charge, that reached the surface was simulated and compared with the experimental results. The plane electrode rotation and radial displacement of the pin one were used to achieve more uniform distribution of the charge on the surface. These setup changes were performed to investigate the minimum charge needed to decontaminate the surface. Subsequent exposition of the same Petri dish was carried out for more precise measurement. The exact electrode position and times are shown in Table 1 and Figure 2.

3. Results and discussion

3.1. Charge dependence

Size of the inhibition zone depends directly on the amount of charge transported to the contaminated agar surface [7]. Two microorganisms (*E. coli* and *S. epidermidis*) were used to test the hypothesis of inhibited zone area dependence on collected charge and current by an analysis of variance (ANOVA) in case of static exposition (i.e. without any movement of a plane electrode). Due to a deformation of the agar surface caused by an ionic wind the discharge current decreases during the treatment. Consequently an initial current and a collected charge can be tested as independent variables by variations of the corona voltage and the treatment duration.

The tested hypothesis were set as

H_0^q the inhibition zone size is not determined by the collected charge only.

H_0^i the inhibition zone size is not determined by the initial corona current only.

H_0^{iq} the inhibition zone is not determined by synergic effect of the collected charge and the initial corona current.

The samples were treated by a negative corona discharge generated with parameters set within the linear part of reduced I-V characteristics, when the charge distribution corresponds to Warburg's law. The results presented in Table 2 show that the inhibition zone area is determined by the collected charge on the agar surface.

Figure 3 shows the dependence of an inhibition area on the charge collected on the contaminated agar surface. There were 15 to 20 repetitions made and the median of decontaminated area was used as a representative result. Increasing trend could be observed for both *S. epidermidis* and *E. coli*.

S. epidermidis is gram-positive bacteria with thicker cellular wall, which was more resistant to plasma treatment. Twice as much charge was needed to reach the same decontamination effect compared to *E. coli*.

Table 2. ANOVA test results.

$\alpha = 0.95$	*S. epidermidis*	*E. coli*
H_0^q	Rejected	Rejected
H_0^i	Not rejected	Not rejected
H_0^{iq}	Not rejected	Not rejected

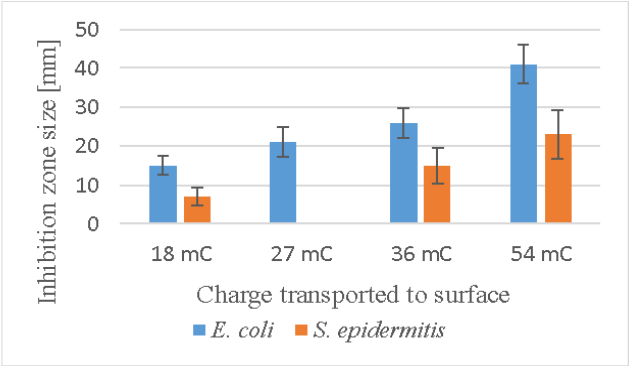


Figure 3. Dependence of the size of decontaminated area on charge transported to the agar surface.

3.2. Setup with rotating electrode

The experiments with rotating plane electrode and the pin electrode with different radial displacement were performed to find out the minimum charge needed to decontaminate the surface [8].

Charge distribution over agar surface was simulated with Wolfram Mathematica software. The minimum value of charge density needed for surface decontamination was estimated to 25 C/m^2 for yeast concentration 10^7 cfu/ml.

Experiments with subsequent expositions per one Petri dish were performed to determine the minimum charge density needed for the yeast inactivation (see Table 1). Results of these experiments can be seen on Figures 4 and 5. Figure 4 shows enlargement of decontaminated zone by increasing the exposition time. Figure 5 shows the results of subsequent expositions. The minimum charge density can be estimated from simulations presented in figures 5c and 5d, where the charge density collected from both expositions is insufficient to inactivate bacteria.

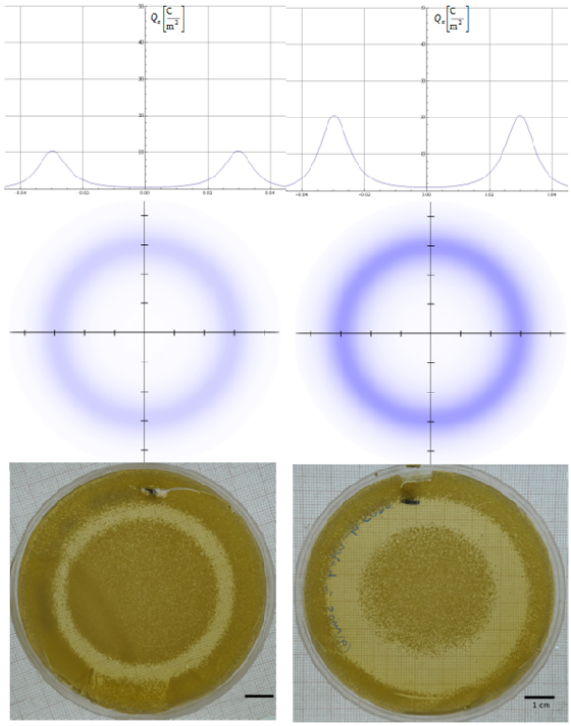
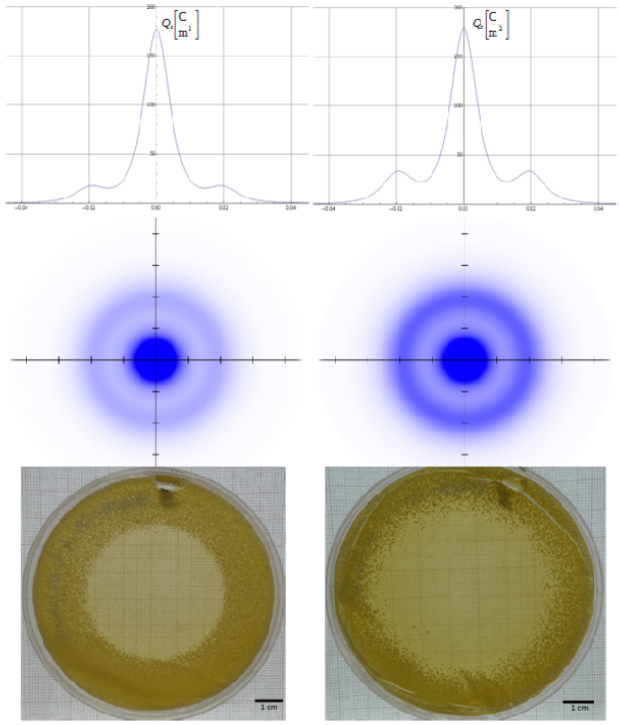
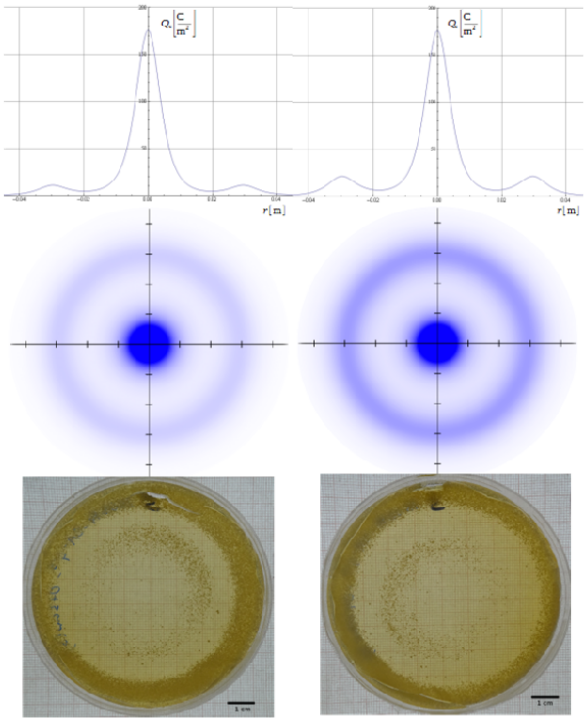


Figure 4. Radial distribution of the surface charge density and corresponding inhibition zones. The radial displacement of the pin electrode for both images was set for 3 cm. The inhibition time was 3 min (left) and 6 min (right).



a. b.



c. d.

Figure 5. Radial distribution of the surface charge density and corresponding inhibition zones for subsequent expositions. The first exposition was for all configurations a, b, c and d done with zero radial displacement of the pin electrode and lasted for 3 minutes. The second one was done immediately after the first one. The radial displacement and time of the second exposition were set as follows: a. 2 cm and 3 min; b. 2 cm and 6 min; c. 3 cm and 3 min; d. 3 cm and 6 min.

Acknowledgement

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4. References

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