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The study of bactericidal effects of corona discharge at atmospheric pressure

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Introduction

Conventional methods of decontamination or sterilization are based on the action of various chemical agents or heat, which may be inapplicable for heat–labile materials, foods etc. It seems possible, that the action of nondestructive non–thermal plasma would appropriately replace the conventional methods. The bactericidal effects of electrical discharges have been previously studied [2], [3], [4], [5]. In this work we present obtained bactericidal characteristics of DC negative corona discharge at atmospheric pressure in air. To study this effects of the non–thermal plasma generated by the corona, we have constructed the simple apparatus generating the corona discharge. Using this apparatus, we examined the bactericidal effects of the corona on various non–sporulating and sporulating bacteria.

Apparatus and method

We have used the apparatus and method described in details in our previous work [1]. Briefly, the cultivating medium was consequently inoculated by defined suspension of bacteria, exposed to the corona discharge, cultivated and evaluated.

The apparatus enables the generation of low–temperature plasma by DC negative point–to–plane corona discharge at atmospheric pressure in air. This discharge was generated on the tip of the cathode electrode, which was the medical needle for hypodermic injection fixed in a laboratory stand. The needle was fixed vertically over the surface of cultivation agar plate; its distance from the surface was adjusted using the screw micrometer. The plane anode electrode was the surface of the cultivation medium.

The following non–sporulating bacterial strains, obtained from the Czech Collection of Microorganisms (CCM), Brno, were used: *Deinococcus radiodurans* CCM 1700, *Enterobacter aerogenes* CCM 2531, *Enterococcus faecium* CCM 3609, *Neisseria sicca* CCM 4404, *Stenotrophomonas maltophilia* CCM 4764 and *Streptococcus sanguinis* CCM 4047. The sporulating species *Geobacillus stearothermophilus* (CCM 4395) was also tried in preliminary experiments.

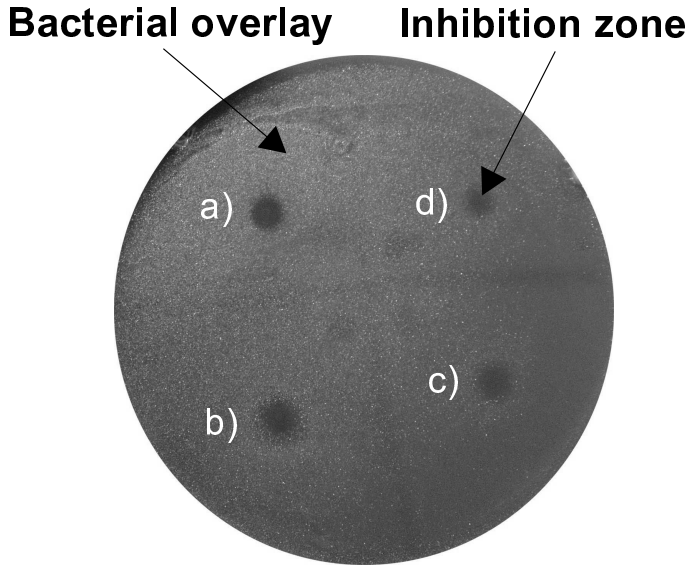

Figure 1: A typical appearence of the bactericidal effect of corona discharge. The inhibition zones are apparent in the continuous overlay of *Streptococcus sanguinis* on the surface of a plate. These zones were induced by the exposition to the discharge for 4 min and the needle distance of 2 mm(a), 4 mm(b), 6 mm(c), 10 mm(d), respectively.

The living cultures were prepared from the freeze–dried samples delivered, diluted to desired concentrations and stored as stock suspensions.

The stock suspensions were applied onto the surface of cultivation agar plates. The plates were inoculated by the suspensions of bacteria at concentrations yielding the continuous bacterial overlay on the plates after cultivation i. e. ca $10^6\,cm^{-2}$ in volume of 1 ml. The inoculated plates were immediately exposed to the corona discharge. The discharge current was adjusted to the constant value 0.05 mA, time of exposition varied for 1, 2, 4, 8 or 16 min. The distance between the tip of the electrode and the agar surface was also variable, being 2, 4, 6, or 10 mm. The exposed plates were incubated at 37 ℃ overnight and the areas of inhibition zones were measured.

Results

All bacterial strains were subjected to the action of corona discharge under above-mentioned experimental condition. As an example, one evaluated plate (actually, the culture of *Streptococcus sanguinis*) is shown in Fig. 1. There are visible the inhibition zones for 4 min exposition to the corona discharge at variable distance of the electrode from plate 2, 4, 6 or 10 mm, respectively.

The results obtained for all examinated bacteria are shown in the Tab. 1–7. As follows from the tables, the area of inhibition zone increases non–linearly with the exposition time. Varying the distance, the area of inhibition zone at longer exposition times (8 and 16 min) appeared for all the electrode distance, whereas small or no inhibition zones appeared at greater distances and shorter exposition times. The results shown for *Geobacillus stearothermophillus* were obtained using the mixture of spores and vegetative cells of this bacterium. The experiments with pure spores showed some encouraging preliminary results; nevertheless, they were not finished yet due to the difficulties with spores preparation and will be published separately.

Table 1: Area of the inhibition zone for *Deinococcus radiodurans* bacteria [mm^2]:

	distance			
time	2 mm	4 mm	6 mm	10 mm
1 min	8	12	6	0
2 min	34	22	9	0
4 min	40	840	41	14
8 min	125	130	110	50
16 min	225	190	180	100

Table 2: Area of the inhibition zone for *Enterobacter aerogenes* bacteria [mm^2]:

	distance			
time	2 mm	4 mm	6 mm	10 mm
1 min	3	3	3	0
2 min	12	12	3	0
4 min	19	62	28	0
8 min	37	62	62	28
16 min	60	80	80	80

Table 3: Area of the inhibition zone for *Enterococcus faecium* bacteria [mm^2]:

	distance			
time	2 mm	4 mm	6 mm	10 mm
1 min	30	30	30	0
2 min	50	50	30	0
4 min	80	110	50	6
8 min	110	110	110	50
16 min	150	150	150	110

Table 4: Area of the inhibition zone for *Neisseria sicca* bacteria [mm^2]:

	distance			
time	2 mm	4 mm	6 mm	10 mm
1 min	12	30	0	0
2 min	40	40	30	0
4 min	60	80	50	30
8 min	130	150	110	60
16 min	150	170	200	200

Conclusion

The corona discharge bactericidal effect was demonstrated on vegetative forms of various bacterial types. It was also characterized quantitatively, showing the dependence on the exposition time and electrode distance. At shorter distances of ca 4 mm, the exposition time of 2–4 min appeared as sufficient for bacterial inhibition.

Table 5: Area of the inhibition zone for *Stenotrophomonas maltophilia* [mm^2]:

	distance			
time	2 mm	4 mm	6 mm	10 mm
1 min	40	40	40	12
2 min	40	60	60	50
4 min	95	110	95	60
8 min	110	125	175	110
16 min	175	175	175	150

Table 6: Area of the inhibition zone for *Streptococcus sanguinis* [mm^2]:

	distance			
time	2 mm	4 mm	6 mm	10 mm
1 min	40	40	30	0
2 min	50	40	30	0
4 min	80	80	80	40
8 min	80	130	130	30
16 min	80	130	130	150

Table 7: Area of the inhibition zone for non–sporulated *Geobacillus thermophilus* [mm^2]:

	distance			
time	2 mm	4 mm	6 mm	10 mm
1 min	12	12	0	0
2 min	12	12	13	0
4 min	18	18	6	0
8 min	30	30	12	3
16 min	50	80	80	50

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