Oxygen plasma inactivation of *Staphylococcus aureus*

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Résumé

The sterilization efficiency of oxygen plasma was studied. Plasma was created in pure oxygen with an inductively coupled radiofrequency discharge. The discharge power was estimated to about 180 W. Plasma parameters were measured with a double Langmuir probe and a catalytic probe. Plasma parameters depended on pressure in the discharge chamber.

Bacteria inactivation effect in low pressure highly dissociated oxygen plasma glow on *Staphylococcus aureus* was studied for various time periods. Sample carriers were treated at pressure of 75 Pa. After the plasma treatment results were obtained by PCT (plate count technique) method - to determine the number of surviving bacteria and eventual sterility of the substrates.

Introduction

Plasma of different origins has been shown to possess effective anti-microbial characteristics [1]. Usage of oxygen plasma represents a simple and safe technique that can be utilized to eradicate unwanted bacteria from different materials [2-3]. Effects of low-temperature oxygen plasma ("cold oxygen plasma") on microorganisms, especially on these bacterial strains which are pathogenic and at the same time often involved in hospitals' infections and food poisoning, need to be further investigated. The aim of this study was to evaluate the inactivation efficiency of bacteria *Staphylococcus aureus* (commonly involved in infections and food poisoning) with low pressure oxygen plasma created by radiofrequency discharge at 27.12 MHz. In order to achieve these aim the plate count technique (PCT) was used to determine the number of surviving bacteria and eventual sterility of the substrates. Despite its shortcomings, the viable plate count is sensitive and has the advantage of only counting living bacteria. Any concentration of microorganism can be easily counted via this method.

The samples of *Staphylococcus aureus* cells were treated in low pressure oxygen plasma for different times, and viable counts of surviving cells were estimated via standard plate counting technique. Samples that had not been exposed to plasma were used as controls. The survivors were counted as colony-forming units (CFUs) per carrier after incubation at 37°C for 24-48 h.

In the first experiment the sample carriers with 1.7×10^8 cells of *Staphylococcus aureus* were exposed to glow discharge. The sterilization conditions were as follows: discharge power 180 W, pressure 75 Pa, the neutral oxygen atom density 3.5×10^{21} m^{-3}, and the density of charged particles 1×10^{16} m^{-3}. The result of oxygen plasma treatment on the survival of the *Staphylococcus aureus* bacteria as a function of plasma treatment time is presented in Figure 1.

The survival curve shows the very straight drop in viability of bacteria up to 5 s of plasma treatment. More than 90% reductions of 1.7×10^8 *Staphylococcus aureus* cells population were observed after 20 s plasma exposure. The final sterilization of sample carriers is achieved after 90 s with an absence of any colony forming unit growth.

To determine the potential influence of bacteria concentration on the plasma inactivation process, two different volumes of 1.7 × 109 initial bacterial cell suspension was used to obtain different cell densities on the glass carrier surface. In the first series of measurements, a 100 µl suspension containing 1.7×10^8 cells were spread on the carrier; in the second series, 150 µl suspension containing 2.55×10^8 cells were used.

The two surviving curves of bacteria don't show the same inclination degree (Figure 2). As expected, the higher concentrations require longer treatment times. The complete inactivation of 1.7×10^8 *Staphylococcus aureus* cells was obtained within 90 s, while at least 120 s was needed to inactivate 2.55×10^8 cells. Therefore, shorter oxygen plasma treatment times are required at sterilization of a lower concentration of bacteria on the glass carriers.

As can be seen from Figures 1 and 2, the number of surviving *Staphylococcus aureus* bacteria cells decreases with an increasing plasma treatment time and reduction in cell viability was achieved.

The plasma sterilization capability demonstrated through this study indicated the potential of this low-presure highly dissociated oxygen plasma as a promising alternative sterilization technique.

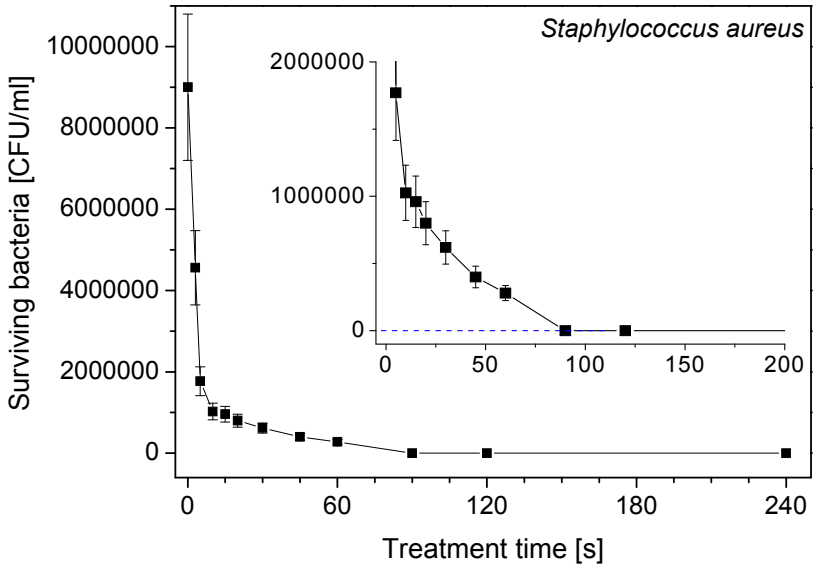

Fig. 1: Survival curve of *Staphylococcus aureus* by PCT. The graph represents CFUs of *Staphylococcus aureus* vs. treatment time by oxygen plasma glow discharge at pressure of 75 Pa. The concentration of bacteria cells on substrate was 1.7×10^8.

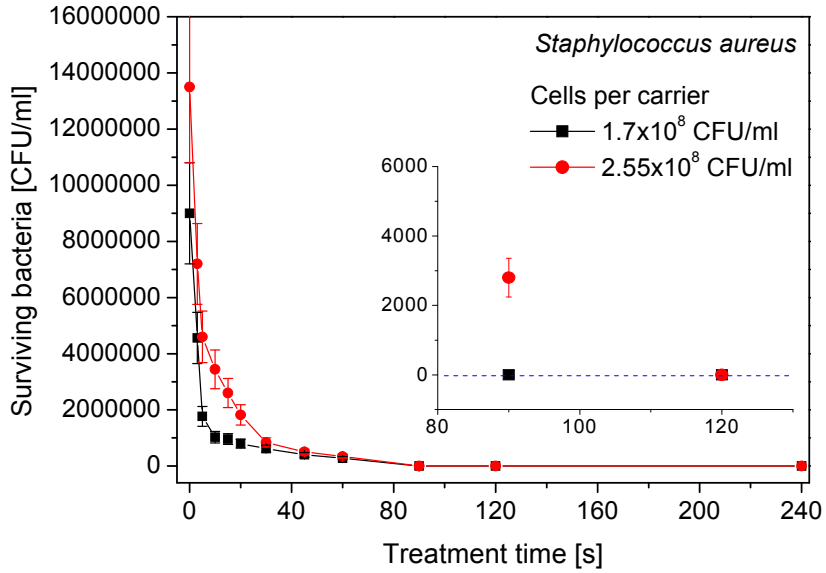

Fig. 2: Survival curves of *Staphylococcus aureus* by PCT. The graph represents CFUs of *Staphylococcus aureus* vs. treatment time by oxygen plasma glow discharge at pressure of 75 Pa obtained with two different concentrations. The concentrations of bacteria cells on substrate were 1.7×10^8 and 2.55×10^8.

References

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