Antimicrobial effect of Al$_2$O$_3$, Ag and Al$_2$O$_3$/Ag thin films on Escherichia coli and Pseudomonas putida

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Abstract. The influence of Al$_2$O$_3$, Ag and Al$_2$O$_3$/Ag thin films on bacterial growth of Gram-negative bacteria Pseudomonas putida and Escherichia coli is studied. The nanostructured thin films are deposited on glass substrates without intentional heating through r.f. magnetron sputtering in Ar atmosphere of Al$_2$O$_3$ and Ag targets or through sequential sputtering of Al$_2$O$_3$ and Ag targets, respectively. The individual Ag thin films (thickness 8 nm) have a weak bacteriostatic effect on Escherichia coli expressed as an extended adaptive phase of the bacteria up to 5 hours from the beginning of the experiment, but the final effect is only 10 times lower bacterial density than in the control. The individual Al$_2$O$_3$ film (20 nm) has no antibacterial effect against two strains E. coli - industrial and pathogenic. The Al$_2$O$_3$/Ag bilayer films (Al$_2$O$_3$ 20 nm/Ag 8 nm) have strong bactericidal effect on Pseudomonas putida and demonstrate an effective time of disinfection for 2 hours. The individual films Al$_2$O$_3$ and Ag have not pronounced antibacterial effect on Pseudomonas putida. A synergistic effect of Al$_2$O$_3$/Ag bilayer films in formation of oxidative species on the surface in contact with the bacterial suspension could be a reason for their antimicrobial effect on E. coli and P. putida.

1. Introduction
The environmental changes or frequent misuse of antibiotics in recent years influence the development of different mutant microbial species resistant to drug treatment. Among the various attempts to surmount the bacterial resistance metal oxide nanoparticles provide encouraging results. It is necessary to study metal oxides, which are chemically stable in different media, exhibiting surface electrostatic charge effect, etc. These properties are promising in bacterial neutralization [1]. The antibacterial activity of nanoparticles depends on their size, surface to volume ratio, environmental pH, etc. The nanoparticles can be prepared in size much smaller than the bacteria. They can penetrate into the cell and attach to important structures inside the bacteria and DNA. Different reactions with bacterial cells are established and are studied for elucidation of nanoparticles - bacteria interaction [2, 3]. The great interest in metal oxide nanoparticles bactericidal effect encounters different challenges concerning to their nature, in particular, their segregation in the solutions, which requires additional care for homogenization of the solutions [4]. Static films of metal oxide are attractive for application in neonatal incubator walls, in sterile rooms, on wound dressing, etc., as an antimicrobial film. Antiseptic
or antimicrobial properties of thin films ZnO and Al\(_2\)O\(_3\) deposited with Atomic Layer Deposition (ALD) method on commercial polymer films typically used for packaging purposes are studied in [5]. Recently, quantum dots of CuO are deposited on glass surface by low-temperature solution process formulated as paint to prepare thin film on glass [6]. The as-coated thin film, thickness about 120 nm, demonstrated high contact bacterial killing effect against Staphylococcus aureus and Escherichia coli. The efficient antibacterial activity is due to intracellular reactive oxygen species (ROS) generated by the CuO quantum dots, which interact with the bacterial cells. This leads to an oxidative attack and finally results in bacterial cell death. The authors propose both contact killing and/or copper ion release killing mechanisms for the antibiofilm activity.

This article presents results for study of the antimicrobial effect of thin films Al\(_2\)O\(_3\), Ag and Al\(_2\)O\(_3\)/Ag on bacterial growth of Gram-negative bacteria Pseudomonas putida and Escherichia coli.

2. Experimental

Thin films Al\(_2\)O\(_3\), Ag and Al\(_2\)O\(_3\)/Ag are deposited on glass substrates without intentional heating during the deposition by r.f. magnetron sputtering of Al\(_2\)O\(_3\) and Ag targets or by sequential sputtering of Al\(_2\)O\(_3\) and Ag targets, respectively. The sputtering atmosphere in the case of Al\(_2\)O\(_3\) is Ar (2 Pa) and in the case of Ag is Ar (0.2 Pa). The sputtering power of Al\(_2\)O\(_3\) and Ag thin film is 50 W and 30 W, respectively. The thickness of Al\(_2\)O\(_3\) films is 20 nm and this of Ag is 8 nm, measured with profilometer Taylor Hobson.

The microorganisms in this study were supplied by the National Bank for Industrial Microorganisms and Cell Cultures and National Center of Infectious and Parasitic Diseases: E. coli 3548 (ATCC 10536) by NBIMCC(industrial strain), E. coli ATCC 25922 by NCIPD (pathogen strain) and Pseudomonas putida 1090 (ATCC 12633) by NBIMCC.

The method for study of thin film antimicrobial effect is inhibition of bacterial growth in dynamic regime. The toxic effect was determined through the classical Koch’s method (plating on solid nutrient medium and counting of survived cells). Two strains of test bacteria E. coli were used - industrial and clinical isolates and one strain of Pseudomonas putida. The control sample didn’t contain any of the studied thin films. The inoculum quantity and nutrient medium in all variants was constant at application on the studied films.

3. Results and discussion

Figure 1 demonstrates the results for the antimicrobial effect of thin films Al\(_2\)O\(_3\)/Ag on the bacterial growth of Ps. putida and the both strains of E. coli. The bacteria Ps. putida were destroyed thoroughly at the second hour from the beginning of the experiment and did not recover till the 24\(^{th}\) hour.

![Figure 1. Antimicrobial effect of thin films of Ag/Al\(_2\)O\(_3\) on bacterial growth of (a) Ps. putida and (b) E. coli, pathogen strain (25922) and industrial strain (3548).](image-url)
The control variant at the same time demonstrated average growth rate of 1.5 x 10^6 cells/hour. From these results an effective disinfection time of 2 hours for Pseudomonas putida in presence of Al2O3/Ag thin film can be determined. The two figures are represented up to the 6th h because during this period the main processes take place.

The industrial and pathogenic strains of Escherichia coli also demonstrate retention in the growth in presence of Al2O3/Ag film figure 1 (b). Strong decrease in the bacterial quantity was observed till the fourth hour – the bacterial colonies were not detected on the nutrient media at this hour by the method of Koch, but only an hour later some colonies appeared and started to grow rapidly. Obviously, a bacterial transformation has occurred and a new resistant mutant colonized the experimental medium.

This result can be explained by neutralization of the dissolved nanoparticles from chelating agents emitted from the bacteria and the rich protein content of nutrient medium. The culture medium for Escherichia coli is rich in organic (meat peptone broth) but the nutrient medium of Pseudomonas putida is entirely synthetic. Obviously, this plays an important role on the antibacterial effect of the thin films. Other fact which could influence the different effects of the thin films on the bacterial growth is that the bacteria belong to different genera, although they have a similar structure of the cell wall.

It can be concluded, that the inhibition effect of the studied thin films on the bacterial growth depends on the composition of the thin films and the test method. The two layer structure Al2O3/Ag has an inhibition effect on E. coli during the first four hours. Similar structure but prepared by impregnation method is studied in [2]. The authors used scavengers for reactive oxygen species (ROS) on the surface of Al2O3/Ag structure and they established no inhibition of the bacterial growth in comparison with the pure Al2O3/Ag structure. This indicates formation of ROS when the Al2O3/Ag structure is in contact with the bacterial suspension. These ROS can be a reason for the bacteriostatic effect. As can be seen from figure 3, the both strains of E. coli have shown some retardation in its development during the first 3-5 hours, but after that due to their adaptation to the environment they begin to grow exponentially till the 24th hour. The measurements at the 24th hour are not represented in the figures for clarity of the cells reaction in the early hours of the bacterial culture development.

Figure 2 displays the growth of Ps. putida and E. coli in presence of thin film Al2O3. An inhibition effect in bacterial development of Ps. putida in the experiment with control and sample (in presence of Al2O3 film) is established between the 2th and 4th hour from the beginning and after the 6th hour the bacterial growth increases till the 24th hour. A prolonged lag-phase till the second hour in the development of E. coli is observed (figure 2 (b)). No significant differences between the both strains of E. coli – clinical and industrial - were established.

Figure 2. Antimicrobial effect of thin films of Al2O3 on bacterial growth of (a) Ps. putida and (b) E. coli, pathogen strain (25922) and industrial strain (3548).
Figure 3 displays the bacterial development in presence of Ag film.

![Graphs showing bacterial growth in control and sample](Image)

**Figure 3.** Antimicrobial effect of thin films Ag on the bacterial growth of (a) *Ps. putida* and (b) *E. coli* industrial (3548) and pathogen (25922) strains.

Retention in the growth during the first 4-5 hours was observed. As the nutrient medium for the inoculum activation and the experiment had the same content and the inoculum was in exponential phase, the delay of bacterial growth was probably due to the influence of the Ag thin film only. It is expected, that metal nanoparticles were eluted from the thin film [4] and have interacted with the cell wall, which decreased the bacteria multiplication rate. The authors of [7] established that in Ag film at ambient conditions exist oxidized silver atoms. The oxide layer on the Ag film could initiate release of Ag⁺, which has antibacterial effect as Ag atoms [8]. Other reason for the antimicrobial activity at the first hours of the experiment could be the morphology of the film – the existence of nanoparticles with different size. It is established [8] a fast release first of small nanoparticles and after that the film is restructured due to formation of film with big nanoparticles which have a lower rate of dissolution and the inhibition effect of the Ag film on the bacterial growth lowers, so the bacteria continue to develop in dependence on the remained nutrient medium [9]. The Ag thin films in this study are deposited on glass substrate by r.f. magnetron sputtering and have columnar structure with grains different in their size [10].

The Ag thin film proved a weak inhibition effect on *E. coli* up to the 4th hour of the experiment, but at the 6th hour the bacterial density in the control and in the sample were similar. This result probably shows that at low concentrations the silver nanoparticles or their ions [4] have a weak effect on the development of *E. coli* and after the 4th hour they adapt to nutrient medium and show exponential growth of cells in the samples (not represented in the figures). According to the displayed graphs the difference between the both bacterial strains of *E. coli* (industrial and pathogen) is so insignificant that it is within the error.

4. Conclusion
Antimicrobial effect of Al₂O₃, Ag and Al₂O₃/Ag thin films on bacterial growth of Gram-negative bacteria *Pseudomonas putida* and *Escherichia coli* is studied. The antimicrobial effect of thin films was determined as inhibition of bacterial growth in periodic culture. The toxic effect was determined through the classical Koch’s method. The Ag thin films have a weak inhibition effect on *Escherichia coli* expressed as an extended adaptive phase of the bacteria up to 5 hours from the beginning of the experiment, but the final effect at the 24th hour is only 10 times lower bacterial density than in the control. This could be due to release of Ag atoms and Ag⁺ ions from the films faster at the beginning of the experiment. The Al₂O₃ thin film has no pronounced inhibition effect against the two strains of *E. coli* - industrial and pathogenic. The individual films Al₂O₃ and Ag have not pronounced antibacterial
effect on *Pseudomonas putida* too. The Al$_2$O$_3$/Ag bilayer films (Al$_2$O$_3$ 20 nm/Ag 8 nm) have strong bactericidal effect on *Pseudomonas putida* with an effective time of disinfection for 2 hours. A synergistic effect of Al$_2$O$_3$/Ag bilayer films in formation of oxidative species on the surface in contact with the bacterial suspension could be a reason for their antimicrobial effect on *E. coli* and *P. putida*.

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