ANTIBACTERIAL EFFECT OF THIN FILMS TiO_2:SiO_2:Ag AGAINST *E.COLI* AND *P.PUTIDA*

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Abstract

The antibacterial effect of thin films TiO_2:SiO_2:Ag on two Gram-negative bacteria *Escherichia coli* and *Pseudomonas putida* was investigated. The thin films were deposited on glass substrates without heating by r.f. magnetron co-sputtering of TiO_2 target with small plates of quartz and Ag on its surface in the zone with maximum erosion. The total area of quartz plates was 750 mm^2 and the area of Ag plates was 40 mm^2 and 100 mm^2. High resistant industrial strain *E.coli* (ATCC 10536) and sensitive *P. Putida* (ATCC 12633) with an ability to form biofilm were chosen for the test. Three experimental techniques were used to estimate the antibacterial activity: classical Koch's method, optical density measurements, and dehydrogenase activity inhibition. The bacteria were cultivated on the studied thin films in a liquid nutrient medium for 24 h without agitation. Complete deactivation of the bacterial growth was observed between the 1st and the 4th hours from the beginning of the experiment. The destruction of the bacterial cells was registered by scanning electron microscope. The developed thin films have a potential for application as antibacterial coating for different medical devices and surfaces.

Keywords: TiO_2:SiO_2:Ag thin films, bactericidal effect, gram-negative bacteria

1. INTRODUCTION

The use of conventional disinfection products and methods have no desired effect in the hospitals and medical devices fail in many cases [1]. Recently, it is observed an increased interest in the application of nanocomposite materials as antibacterial surfaces, which combine mechanical stability and biocompatibility. Different nanomaterials have multi-targeted mechanism of action on various microorganisms, which are danger for the human health. Contemporary industrial technologies in material production provide control of nanostructured dimensions and surface morphology of nanomaterials with antibacterial activity [2,3].

TiO_2 is a widely used for scientific studies and it finds an application in different fields, like modern oral implants, textile impregnation, ect. [3, 4, 5]. The properties of TiO_2 could be improved through introduction of suitable metals in the structure of TiO_2 [6-7]. In addition, the presence of dopant could increase the adhesion and mechanical stability of the thin film on substrates which play a key role in device reliability [8]. Silver doped TiO_2 was extensively studied due to its wide applications in environmental remediation, catalytic oxidation reactions,antimicrobial protection etc. [9-10].

Eker et al., studied TiO_2 coated plexiglass surface and observed bacterial reduction of 25% and 48% after 1h and 2h illumination,respectively. The reduction was increased up to 68% at Ag-TiO_2 coated plexiglass, evidently due to the presence of Ag. These results indicate that such Ag-TiO_2 thin film could be of interest for antimicrobial protection of incubators by plexiglass [11].

Rilda et al., [12] prepared TiO_2-SiO_2 coated cotton textile by cross-linking with an acrylic acid compound. The clusters of TiO_2-SiO_2 were modified by different Ti: Si molar ratios of compositions. The best self-cleaning effect of the coated cotton was achieved at Ti: Si molar ratio of 1:2.

As far as we know, no studies of thin films TiO_2:SiO_2 :Ag obtained by magnetron co-sputtering were conducted. The magnetron sputtering is considered as one of the most effective processes for the deposition of a wide range of thin films [13-14]. Therefore the aim of this investigation was the magnetron co-sputtering preparation of TiO_2:SiO_2:Ag thin films with different Ag content and evaluation of antibacterial activity.

2. MATERIALS AND METHODS

Preparation of thin films: The thin films were deposited on glass substrates without heating by r.f. magnetron co-sputtering of a TiO_2 target (99.99 % purity and diameter of 100 mm, supplied by Kurt J. Lesker Company) with small plates of quartz and Ag on its surface in the zone with maximum erosion. The total area of quartz plates was 750 mm^2 and the areas of Ag plates were 40 mm^2 and 100 mm^2 in two different kinds of samples. The thickness of the films measured with Taylor Hobson profilometer was about 100 nm and 200 nm.

Microorganisms: The microorganisms for this study: *E. coli* ATCC 10536 and *Pseudomonas putida* ATCC 12633 were supplied by the National Bank for Industrial Microorganisms and Cell Cultures (NBIMCC).

Antimicrobial activity: The bacterial growth was conducted in six-well plates. Optical density and survived cells were determined in different time points as it was described earlier [15]. TTC (triphenyl tetrazolium chloride) and INT (Iodonitrotetrazolium chloride) were used as electron acceptors to measure metabolic inhibition [16].

Atomic Flame Absorption Spectrometry (Perkin Elmer Analyst 400) was used for determination of Ag in the cultural liquid and control medium. The total metal content (nanoparticles and ions) was determined at the 24th h of the experiment.

Scanning Electron Microscope, JOEL model JSM 5510 (Japan) was used for observation of the bacterial surface morphology on the thin films.

3. RESULTS AND DISCUSSION

The complete inactivation of the Gram-negative pathogen, *E. coli* is presented in **Figure 1** (**A** and **B**). The inhibition of the bacterial growth was observed by the Koch's method (**Figure 1A**) and optical density (**Figure 1B**).

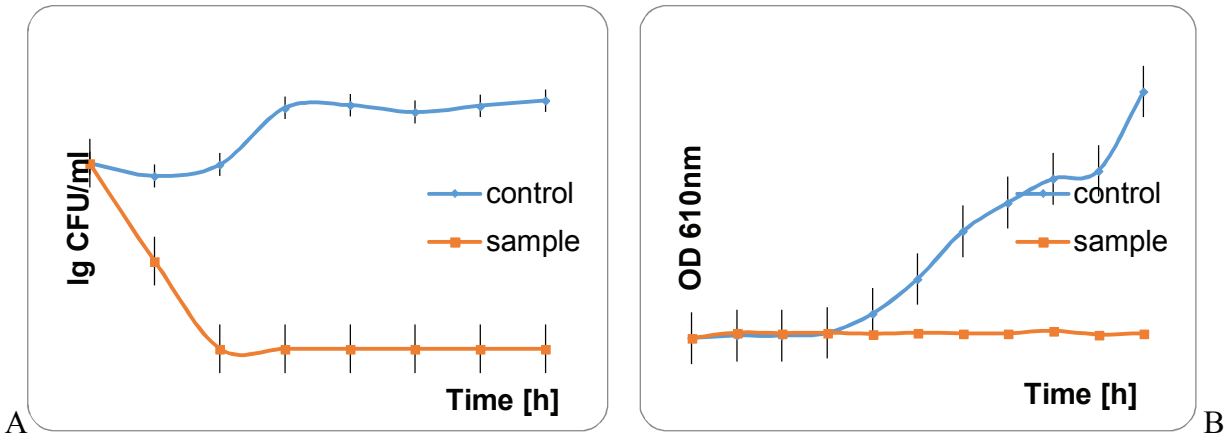

Figure 1 Growth of *E. coli* with (red line) and without TiO_2:SiO_2:Ag (Ag area of 40 mm^2) and 100 nm thin films (blu line) presented by lgCFU/ml (A) and optical density measurements (B)

The loss of viability of *E. coli* was established at the second hour from the beginning of the experiment, as can be seen from **Figure 1**. The thin film (S_{Ag}=40mm^2; thickness - 100nm) was characterized with fast dissolution of Ag atoms in the nutrient medium and this fact could explain the values of the measured optical density in the sample. The dissolved nanoparticles and ions cause a rapid bactericidal effect on industrial *E. coli.* The effect of the same thin films on the *P. putida* is presented in **Figure 2A** and **2B**. In this case, slower bactericidal effect (till the 4th hour) and no survived cells after 24th h were observed.

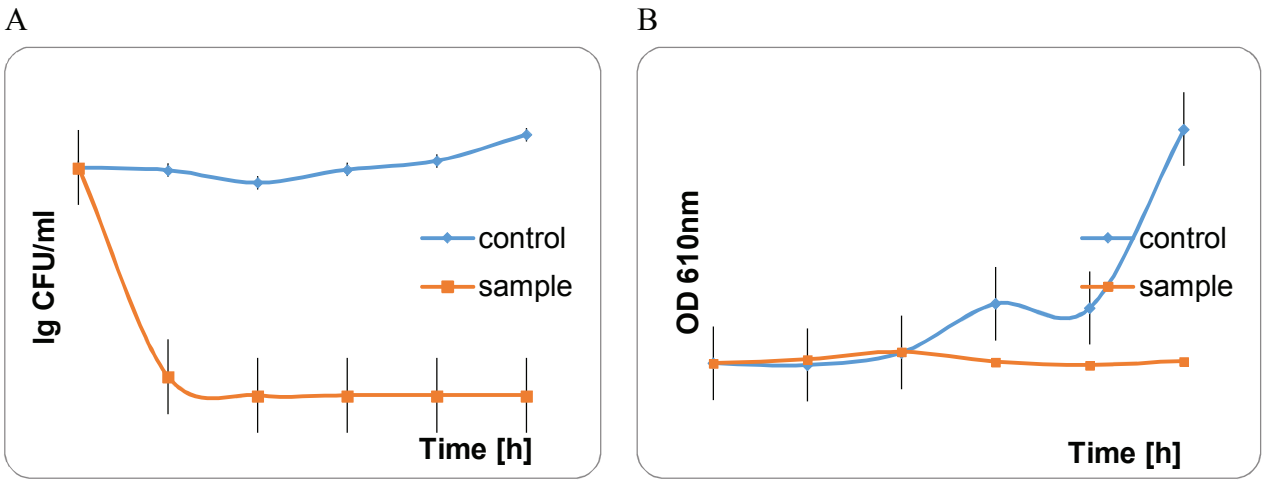

Figure 2 Growth of *P. putida* with (red line) and without TiO_2:SiO_2:Ag (Ag area of 40 mm^2) and 100 nm thin films (blu line) presented by decimal logaritm of CFU/ml (A) and optical density measurements (B)

Thin films obtained through co-sputtering of films at 100 mm^2 total area of Ag and with thickness of 200 nm, demonstrated bactericidal effect on *E.coli* at the first hour after the treatment (**Figure 3**).

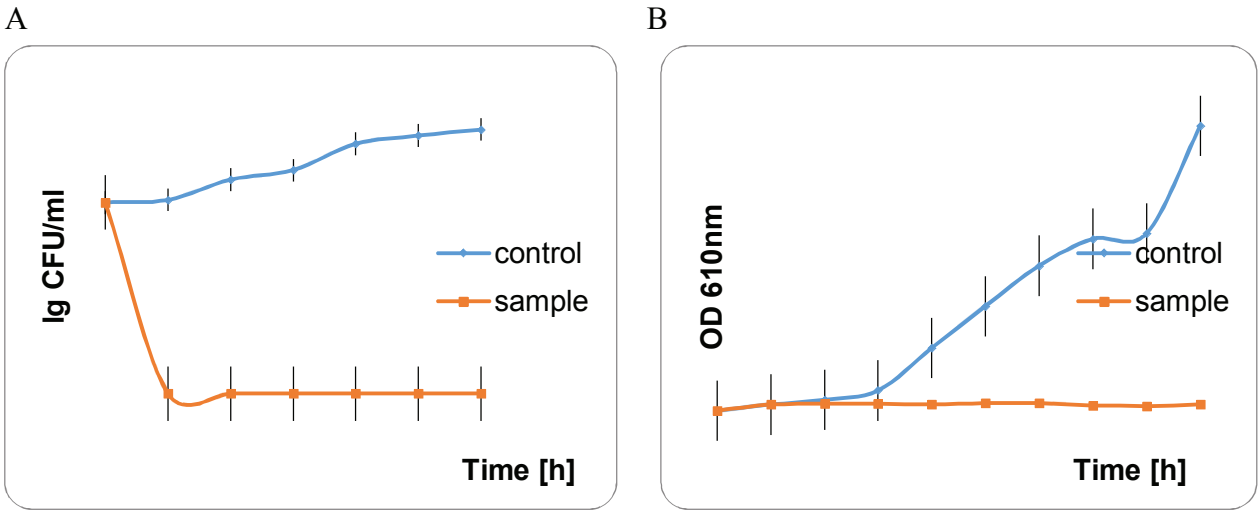

Figure 3 Growth of *E. coli* with (red line) and without TiO_2:SiO_2:Ag (Ag area of 100 mm^2) and 200 nm thin films (blu line) presented by lg CFU/ml (A) and optical density measurements (B)

The same material caused bactericidal effect on *P. putida* in the 1st hour of the exposition according to Koch's method (**Figure 4A**) and in the 2nd h by the optical density measurements (**Figure 4B**).

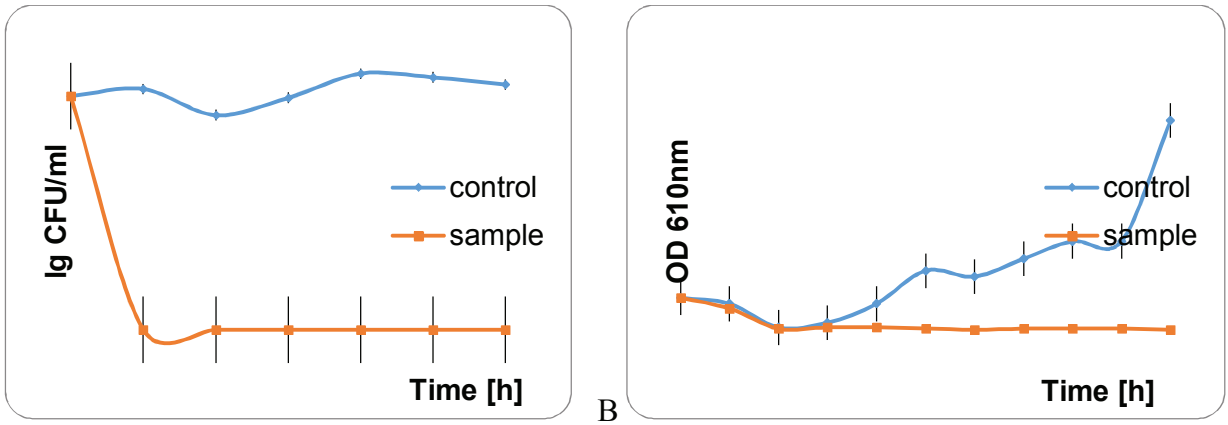

Figure 4 Growth of *P. putida* with (red line) and without TiO_2:SiO_2:Ag (Ag area of 100mm^2) and 200nm thin films (blu line) presented by lgCFU/ml (A) and optical density measurements (B)

It is clear from the graphs that both thin films have a similar bactericidal effect on *E. coli*. The other selected test bacteria - natural isolate *P. putida* was characterized with a higher sensitivity to nanosized material with a layer thickness of 200 nm and a silver area of 100 mm^2. In this case the bactericidal effect was observed at the 1st h.

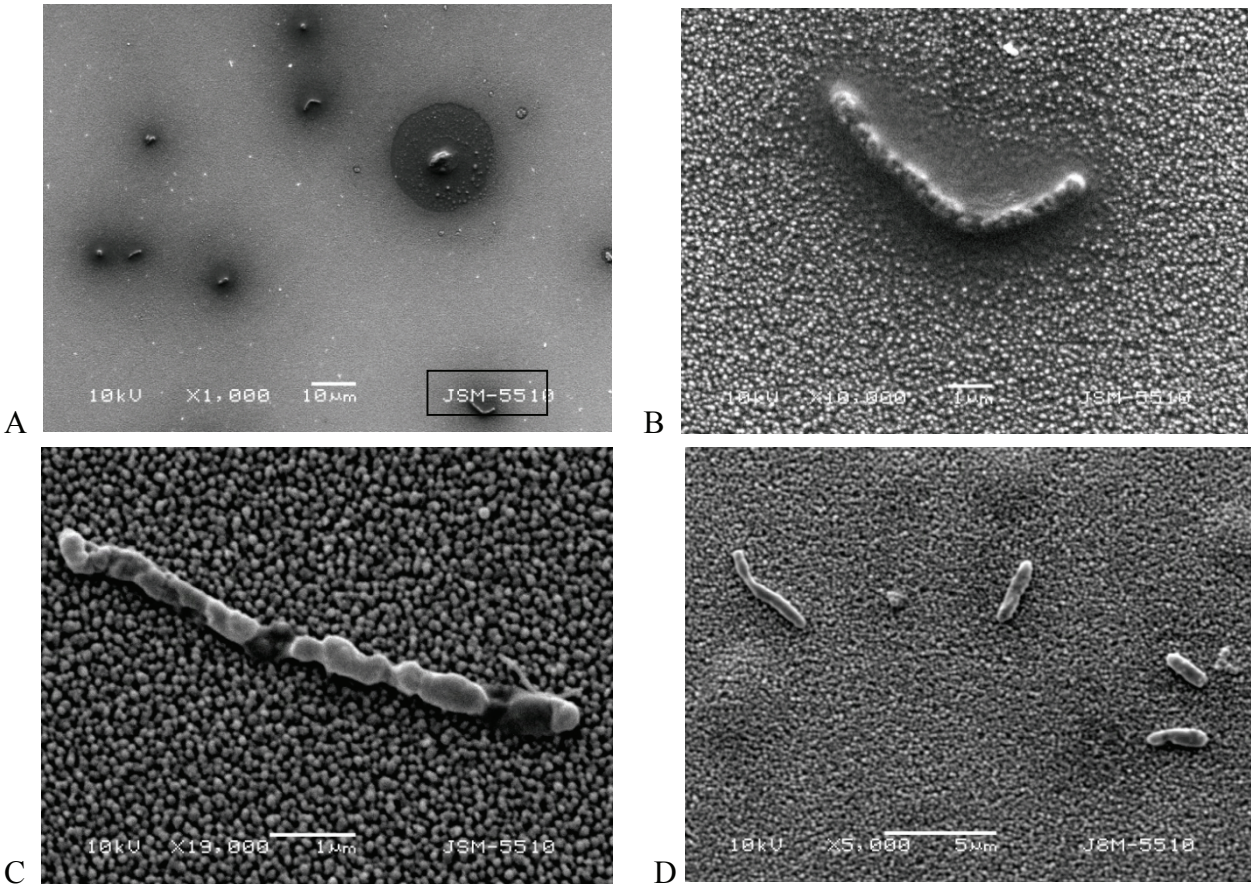

Figure 5 The images of the tested bacterial cells by Scaning Electron Microscope (A) and (B) - *E. coli* on 100nm thin film TiO_2:SiO_2:Ag; (B)- Magnified image of the marked area in 5 A; (C) - *P. putida* on the 100 nm thick and (D) - *P. putida* on the 200 nm thick TiO_2:SiO_2:Ag thin film

The micrograph images show an extended form of the bacteria, probably due to the fact of membrane and important macromolecules damages that hinder cell division.

The dark zones around the cells of *E. coli* (**Figure 5A** and **5B**) are cell content leakages burned by the electron beam or organic nutrient medium. The samples were not washed before drying. In contrast, *P. putida* was characterized by morphological destruction only, as it was cultivated in synthetic nutrient medium. This fact suggested the different mechanism of thin films action on the tested bacteria. The toxic effect was confirmed by an assessment of the bacterial dehydrogenase activity. As it's well known this activity is a proof of normal cell metabolism and its inhibition is due to a destruction of the bacterial cell wall and membrane. At the control (bacteria without thin films) was established dehydrogenase activity in a range of 0.048 FNU for *E. coli* and 0.01 FNU for *P. putida* respectively. In presence of both thin films (100 and 200 nm thickness) no dehydrogenase activity was detected.

The established concentration of Ag (nanoparticles and ions) in control and sample was measured by Atomic flame Absorption Spectrometry. The values for the blank control (organic and synthetic nutrient media with a thin film without bacteria) were determined as 1.3 mg/L Ag for a 100 nm thin film in the organic nutrient medium and as 4.2 mg/L in the synthetic nutrient medium. The difference is due to the slightly acidic pH of synthetic nutrient medium for *Pseudomonas* medium. In the presence of *E. coli* and *P. putida* the dissolved total Ag were 0.95 mg/L and 1.65 mg/L respectively. Obviously the difference in the silver content in the control without bacteria and in sample with bacteria is due to bacterial absorption.

In the case of 200 nm thin film, the value for the blank control was determined as 4.3 mg/L in the organic nutrient medium and 4.6 mg/L in the synthetic nutrient medium. The same thin films in the presence of *E. coli* and *P. putida* demonstrated dissolution of 3.4 mg/L and 3.8 mg/L respectively.

4. CONCLUSION

Different inhibition of the bacterial growth on TiO_2: SiO_2: Ag thin films with different content of Ag and layer thicknesses (100 nm and 200 nm) was observed. The reaction of bacteria to the thin films was specific and dependent on their taxon. Both bacteria, *E. coli* and *P. Putida,* were killed in a short time. Considering that the used strains are more resistant than pathogenic ones, one could suggest the possible use of thinn films for antibacterial surface coatings.

ACKNOWLEDGEMENTS

Sofia University Scientific Fund (project 80-10-12/12.04.2017 and BG05M2OP001-2.009-0013) and Bulgarian National Scientific Fund (grand DCOST 01/14/08.12.2016) are gratefully acknowledged for the financial support of this investigation performed in the frame of COST Action TD 1305 "Improved Protection of Medical Devices Against Infections".

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