Antimicrobial effect of TiO\textsubscript{2} doped with Ag and Cu on Escherichia coli and Pseudomonas putida

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Abstract. Antimicrobial effect of TiO\textsubscript{2} doped with Ag and Cu on Gram-negative bacteria Escherichia coli and Pseudomonas putida is studied. The thin films are deposited on glass substrates without heating during the deposition by r.f. magnetron co-sputtering of TiO\textsubscript{2} target and pieces of Ag and Cu. The studied films, thickness about 65 nm, were as deposited and annealed (520\textdegree C, 4h, N\textsubscript{2}+5\%H\textsubscript{2}, 4Pa). The as deposited thin films TiO\textsubscript{2}:Ag:Cu have band gap energy of 3.56 eV little higher than the band gap of crystalline anatase TiO\textsubscript{2} which can be explained with the quantum effect of the granular structure of r.f. magnetron sputtered films. The annealed samples have band gap of 2.52 eV due to formation of donor levels from Ag and Cu atoms near the bottom of the conduction band. The toxic effect was determined through the classical Koch’s method and the optical density measurements at \(\lambda=610\) nm. The as deposited TiO\textsubscript{2}:Ag:Cu thin films demonstrate stronger inhibition effect - bactericidal for \textit{P. putida} and bacteriostatic for \textit{E. coli} (up to the 6\textsuperscript{th} hour) in comparison with the annealed samples. The both methods of study show the same trends of the bacterial growth independently of their different sensitivity which confirms the observed effect.

1. Introduction
TiO\textsubscript{2} is a widely used material for scientific study and application in different fields due to its high stability at chemical treatments and environment influence. Transition metals (W, V, Ag, Cu, etc.) or non-metals (S, C, N\textsubscript{2}) doped TiO\textsubscript{2} has lower band gap than pure TiO\textsubscript{2} and can be used to increase the production of reactive oxygen species generated under visible light irradiation in solutions containing undesired pollutants [1, 2]. This modification of TiO\textsubscript{2} band gap doesn’t affect its ultraviolet light activity and increases the photocatalytic activity at sunlight. TiO\textsubscript{2} can be successfully applied as antimicrobial agent for deactivation of different microorganisms in waste water, wound dressings, medicine, incubator walls for the neonatology, etc., [3]. Surface to volume ratio of the nanomaterials plays an important role in application of TiO\textsubscript{2} so the material is mainly in the form of nanoparticles which are usually dispersed in solution. However, this leads to segregation of the nanoparticles in dependence on their sizes, mass, temperature, pH and other environmental characteristics, which requires stabilization and homogenization of the nanoparticles [4, 5]. This invokes the necessity for synthesis and study of different structures of TiO\textsubscript{2} for optimization of its application as bactericide agent. Akhavan has studied the role of Ag-TiO\textsubscript{2}/Ag/a-TiO\textsubscript{2} nanocomposite film prepared by sol-gel method for photodegradation of Escherichia coli [6]. He established OH bounds and H\textsubscript{2}O contents on...
the film surface and at the interface between layers. These species stimulate the release of more ionic silver than Ag atoms due to inter-diffusion of H2O and Ag nanoparticles through pores of the TiO2. The Ag+ are responsible for the antibacterial effect of this nanocomposite thin film structure. The authors of [7] compared antimicrobial activity of TiO2/Ag, TiO2/Cu and co-deposited TiO2-CuO with single layers. They established good antibacterial effect of Cu-TiO2 films under irradiation through photo-Fenton type reaction (creation of free radicals via intracellular reactions of chemicals which are present in vivo). Thin films TiO2 on surfaces in sterile zones, incubator walls, etc., could provide static antimicrobial cover, which is durable and active for a long time [8].

The aim of this article is to compare the antimicrobial effect of TiO2 thin films doped with Ag and Cu on Escherichia coli and Pseudomonas putida.

2. Experimental

Thin films TiO2 are deposited on glass substrates without deliberately heating during the deposition by r.f. magnetron co-sputtering (13.56 MHz) of TiO2 target and placed together Ag and Cu pieces on the target surface in its maximum erosion zone. The total surface area of Ag (4 alike pieces) is 40 mm² and this one of Cu (4 alike pieces) is 80 mm². The r.f. power is 50 W and the sputtering atmosphere is Ar (0.8 Pa). The thickness of the films is about 65 nm and is measured with profilometer Taylor Hobson. The studied films were as deposited and annealed in N2+5%H2 atmosphere, 4 Pa, at 520°C for 4 h. The optical properties of the films were analyzed through transmittance and reflectance measurements. The structure of the films were characterized by Raman spectroscopy.

The microorganisms in this study were supplied by the National Bank for Industrial Microorganisms and Cell Cultures (NBIMCC): E. coli 3548 NBIMCC (ATCC 10536) and Pseudomonas putida 1090 (ATCC 12633) by NBIMCC.

The thin film antimicrobial effect was studied by observation of bacterial growth in periodic culture. The toxic effect was determined through the classical Koch’s method (plating on solid nutrient medium and counting of survived cells) and the optical density measurements (OD) at λ=610 nm. During the experiment through the Koch’s method the control and samples were irradiated with a lamp Vivalux of 20 W at a distance of about 10 cm. The experiment for OD measurements was conducted in a six-well plastic plate. Series of two replicates for the controls with bacteria and pure glass without thin film, three replicates for tested thin films and bacteria and an empty control only with nutrient medium and a thin film were prepared. The inoculum quantity in all variants was constant. The optical density was measured every hour and in every 2 hours consecutive decimal dilutions were prepared for determination of the bacterial quantity of the samples and the controls. Each dilution is done in duplicate. The measurement of optical density was carried out with spectrophotometer Spekol 11 at λ=610 nm every hour up to 24 hours.

3. Results and discussion

Figure 1 displays the dependence of (α.E)0.5 (absorption coefficient α, multiplied by photon energy, E) on energy for as deposited and annealed thin films TiO2:Ag:Cu. The absorption coefficient is calculated through transmittance and reflectance spectra in the energy range corresponding to the fundamental absorption [9]:

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α = \frac{(1/d)\ln[(1-R)^2/T]}{E^0.5}
\]

where α is absorption coefficient, d-film thickness, [cm], R-reflectance and T-transmittance.

The energy band gap is determined by the Tauc’s equation [10] for indirect electron transitions typical for TiO2. The band gap of the as deposited TiO2:Ag:Cu thin films determined by the cross point between the tangent to the plot at the range near the absorption edge and the coordinate axis of energy, E, is about 3.56 eV, little higher than the band gap of anatase TiO2 (~ 3.2 eV). This can be explained with the quantum size effect [11] due to granular structure of amorphous TiO2 deposited by r.f. magnetron sputtering. At annealing the Ag and Cu atoms coalesce through diffusion inside the layer
and create nanoparticles with different sizes and shapes because the interaction between the metal atoms is higher than between the individual atoms and TiO$_2$ layer. Raman spectra (not shown) demonstrate amorphous structure of the as deposited and annealed films. In the transmittance spectrum (not shown) of annealed samples there is a dip narrow band centered at about 400 nm. This band is ascribed to intensive plasmon resonance of Ag nanoparticles due to overlap between quadrupole and dipole resonance [12]. The position of this band depends on the size of nanoparticles and dielectric function of the TiO$_2$ layer. At wavelengths lower than 400 nm the transmission of the annealed samples is due to intensive quadrupole resonance of the Ag nanoparticles. The quadrupole resonance emits at about 365 nm stronger in forward direction, thus the signal from the sample is registered by the detector as transmission of the sample. At these conditions the denominator of the equation (1) increases the nominator is low and the logarithm is not defined with argument near to 0. This range corresponds to the discontinuity in the dependence of the absorption coefficient $\alpha$ on energy, $E$ (3.68-3.9 eV). The band gap of the annealed TiO$_2$:Ag:Cu films is 2.52 eV ($\lambda=492$nm) due to formation of donor centers of the doping transition metals Ag and Cu atoms in the band gap near the bottom of the conduction band. This leads to visible light absorption of the TiO$_2$:Ag:Cu films which is potential for improvement of their UV and visible light photocatalytic activity.

Figure 2 demonstrates the bactericidal effect of as deposited thin films TiO$_2$:Ag:Cu on *P. putida* growth, determined by the Koch’s method (plating on solid nutrient medium and counting of the survived cells), (a), where the black line presents the bacterial quantity for the control without TiO$_2$:Ag:Cu film and the red line represents the quantity of survived cells in sample with TiO$_2$:Ag:Cu film, both in logarithm scale and (b) the results by the optical density measurements at $\lambda=610$ nm. The as deposited TiO$_2$:Ag:Cu films demonstrate no bacterial growth after the 2nd hour from the beginning. This effect on bacterial development continues till the end of the experiment at the 24th hour. In the experiment with the control (the same nutrient medium and bacteria CFU/ml as in the experimental sample but without TiO$_2$:Ag:Cu thin film) the bacterial growth increases up to the 10th hour from the beginning and after that it decreases till the end of the experiment at the 24th hour. This can be explained with the depletion of the nutrient medium. The same trend is observed in the results obtained by the method of optical density measurements. The both methods which have different sensitivity level demonstrate similar trends in inhibition of the development of bacteria in presence of TiO$_2$:Ag:Cu the films and prove the bactericidal effect of the as deposited TiO$_2$:Ag:Cu thin films on *P. putida*. 

![Figure 1. Dependence of $(\alpha E)^{0.5}$ on photon energy, $E$, for TiO$_2$:Ag:Cu, as deposited – red line and annealed – black line.](image-url)
Figure 3 demonstrates the antimicrobial effect of annealed thin films TiO$_2$:Ag:Cu on the growth of bacteria $P. \text{putida}$ by the method of Koch, (a), and optical density measurements, (b), at $\lambda=610$ nm. The both methods of study the antibacterial effect on $P. \text{putida}$ demonstrate inhibition of the bacterial growth from the beginning till the end of the experiment at the 24$^{th}$ hour. The annealed samples demonstrate weaker inhibition effect on the development of $P. \text{putida}$ by the both methods in comparison with the as deposited films.

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The control (without TiO$_2$:Ag:Cu thin film) demonstrates a decrease in the growth because of the depletion of nutrient medium after the exponential and stationary phase of bacterial growth (up to the 8th hour) obtained by the Koch’s method. There is the same trend in inhibition of the bacterial growth in the sample till the 3rd h and an increase in bacterial growth after it according to the results obtained by the optical density measurements (figure 4 (b)). This can be explained with the different sensitivity and timing of the Koch’s method and the method of the optical density measurement.

In figure 5 are presented the results for the antimicrobial effect of annealed thin films TiO$_2$:Ag:Cu on the bacterial growth of *E. coli*.

The both methods used for determination of the effect of the annealed films demonstrate no inhibition effect on the bacterial growth and the same trends of increasing the bacterial quantity in comparison with the results of as deposited thin films (figure 5).

The annealed samples demonstrate intensive plasmonic absorption at about 400 nm under illumination but this didn’t lead to increase in the antimicrobial effect. This can be explained with the influence of doping Ag and Cu atoms on the process of recombination of the generated electrons under illumination as recombination centres of transition metals inside TiO$_2$ [13]. The defective states in amorphous TiO$_2$ deposited by r.f. magnetron sputtering are responsible for photocatalytic inactivity of
the film [14]. This leads to loss of electrons from the bulk. Such electron-holes cannot reach the surface of TiO$_2$:Ag:Cu and cannot react with the attached to the film bacteria for creation of reactive oxygen species (ROS). Thus, small amount of electron-holes generated on the surface of TiO$_2$:Ag:Cu could participate in formation of ROS. The authors in [15] are established the necessity for the film thickness to be higher than a threshold value for good photocatalytic properties of amorphous TiO$_2$ doped with transition metals. The mechanism of antibacterial effect of as deposited thin films TiO$_2$:Ag:Cu maybe is due to metal ions toxicity to bacteria or to mechanistic demolition of cell wall by small nanoparticles, but is not due to formation of reactive oxygen species as established in [16].

4. Conclusion
The antimicrobial effect of TiO$_2$ doped with Ag and Cu on Gram-negative bacteria *Escherichia coli* and *Pseudomonas putida* is studied. The as deposited thin films TiO$_2$:Ag:Cu are amorphous and have band gap energy of 3.56 eV little higher than the band gap of crystalline anatase TiO$_2$ which can be explained with the quantum effect of the granular structure of r.f. magnetron sputtered films. The annealed samples have band gap of 2.52 eV due to formation of donor levels from Ag and Cu atoms near the bottom of the conduction band of the films. The as deposited TiO$_2$:Ag:Cu thin films demonstrate bactericidal effect on *P. putida* and an inhibition effect (up to the 6th hour) on *E. coli*, which is stronger in comparison with the annealed samples. The both methods of study the antimicrobial effect show the same trends of the bacterial growth independently of their different sensitivity. It is necessary to elucidate the mechanism of antibacterial action of as deposited thin films TiO$_2$:Ag:Cu. For the annealed thin films TiO$_2$:Ag:Cu we can conclude that it is necessary to elucidate the possibility for improvement of antibacterial effect and their plasmonic properties through variation of Ag and Cu content as dopants in TiO$_2$ thin films.

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