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Bacterial adhesion and inactivation on Ag decorated TiO_2-nanotubes under visible light: Effect of the nanotubes geometry on the photocatalytic activity

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ABSTRACT

This study investigates the effect of the diameter of TiO_2 nanotubes and silver decorated nanotubes on optical properties and photocatalytic inactivation of *Escherichia coli* under visible light. The TiO_2 nanotubes (TiO_2-NTs) were prepared using the electrochemical method varying the anodization potential starting from 20 V until 70 V. The Ag nanoparticles were carried out using the photoreduction process under the same experimental conditions. The diameter size was determined using the scanning electronic microscopy (SEM). TiO_2-NTs diameter reached ~100 nm at 70 V. Transmission electronic microscopy (TEM) imaging confirmed the TiO_2-NTs surface decoration by silver nanoparticles. The Ag-NPs average size was found to be equal to 8 nm. The X-Ray diffraction (XRD) analysis confirm that all TiO_2-NTs crystallize in the anatase phases regardless the used anodization potential. The decrease of the photoluminescence (PL) intensity of Ag NPs decorated TiO_2-NTs indicates the decrease of the specific area when the nanotubes diameter increases. The UV–vis absorbance show that the absorption edges was bleu shifted with the increasing of nanotubes diameter, which can be explained by the increase of the crystallites average size. The bacterial adhesion and inactivation tests were carried in the dark and under light. Bacteria were seen to adhere on TiO_2-NTs in the dark; however, under light the bacteria were killed before they establish a strong contact with the TiO_2-NTs and Ag/TiO_2-NTs surfaces. Bacterial inactivation kinetics were faster when the anodizing potential of the NTs-preparation increases. A total bacterial inactivation was obtained on ~100 nm nanotubes diameter within 90 min. This result was attributed to the enhancement of the TNTs crystallinity leading to reduced surface defects. Redox catalysis was seen to occur under light on the TiO_2-NTs and Ag/TiO_2-NTs. the photo-induced antibacterial activity on the AgO/Ag_2O decorated TiO_2-NTs was attributed to the interfacial charge transfer mechanism (IFCT).

1. Introduction

Escherichia coli (*E. coli*) is hazardous bacteria that can cause distinct diarrhea syndrome. it was recognized as pathogen because it can cause urinary tract infection, hemolytic uremic syndrome and thrombotic thrombocytopenic purpura in human [1]. Thus, it is necessary to develop an effective material for the inactivation of *E. coli*.

Currently, the inactivation of bacteria is mainly through chemical products based on chloride as strong oxidant. Unfortunately, a big majority of these poducts produce carcinogenic disinfection by-products (DBPs) like trihalomethanes and haloacetic acids [2]. Oher products are based on bactericidal nanoparticles (mainly silver). These later products are showing limitations towards some germs. In addition, some pathogens can develop resistance to silver nanoparticles as recently reported [3]. World Health Organization (WHO) launched an alert towards the excessive use of antibiotics and their derivatives [4]. Antibiotics are today showed to become ineffective against many pathogens that are able to develop resistance; hence, many germs became multidrug resistant. Thus, it is necessity today to develop alternative ways to disinfect surfaces in hospital facilities since healthcare acquired infections are increasingly threatening our lives [5–7].

Photocatalytic bacterial inactivation is found to be a promising alternative due to several advantages. The sustainable use of the photocatalytic material is one of the most unique properties of this method while conventional chemical root consumes disinfectants [8]. also this new method does not produce any toxic or carcinogenic product (DBPs). Additionally, Titanium dioxide (TiO_2) is attracting great attention during the last decades because of its chemical stability, non-toxicity, high oxidative power and low fabrication cost [5]. Titanium based photocatalysts have been used to inactivate bacteria (in the dark

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or under light irradiation) [9,10], virus [11] and even cancer cells/tumor [3–6]. However, the wide band gap energy (3.2 eV) and the high recombination rate of the photo-generated charges in TiO_2 reduces its photocatalytic performance. To overcome this limitation, many strategies can be adopted like modifying TiO_2 with anionic or cationic doping [12,13], narrow-band semi-conductors coupling [14] or metallic nanoparticles decoration [15]. Although, it has been reported since years that anatase and rutile crystalline phases of TiO_2 are more active than brookite form [16]. With respect to the photocatalytic activity of the polymorphs, although it is somewhat controversial, the anatase phase is generally regarded as being more active than rutile [17,18]. An emerging field of interest in photocatalysis is the development of TiO_2 nanotubes (TiO_2-NTs) and their coupling with cations, metal-oxides and additional composites leading to a higher nanocomposite sensitization in the visible range [19–21]. Titanium anodization is a simple preparation leading to controllable nanotubes formation, chemical resistivity, high surface area (2–3 orders of magnitude higher than a flat surface) and therefore high loading capability. The large surface to volume ration of TiO_2-NTs is related to the large internal and external surfaces along with the surface in their vertex and the interstitial surface.

In this study, TiO_2-NTs were prepared by anodic oxidation. After optimization of the TiO_2-NTs antibacterial activity and geometry, the most active NTs were decorated with different amounts of silver nanoparticles (Ag NPs) using the photo-reduction method. We focus on the effect of NTs geometry before and after the Ag-decoration and its influence on the charge separation and transport. Furthermore, this decoration affected the optical properties and photocatalytic performance against *E. coli* under low intensity solar simulated light. We also show that varying the anodization potential during the nanotubes growth influences their geometry and reactivity under visible light.

2. Experimental

2.1. Preparation of Ag-NPs/TiO_2-NTs photo-electrodes

The anodization was performed under continuous stirring during 2 h at room temperature and at different potential starting from 20 V to 70 V. The electrolyte consisted of a mixture of 2 vol.% of water and ethylene glycol and 0.07 M of NH_4F. The as-prepared TiO_2-NTs were rinsed with water, air dried, and then annealed for 3 h at 400 °C (5 °C/min). Subsequently, the prepared TiO_2-NTs were decorated with Ag-NPs using the photo-reduction method. To this end, the TiO_2-NTs were first immersed in a 0.1 M solution of $AgNO_3$ for 24 h, then rinsed with water and immersed again in methanol under UV radiation (254 nm) for 10 min. Finally, the Ag-NPs/TiO_2-NTs samples were rinsed with water and dried in a vacuum oven for 1 h at 80 °C.

2.2. Evaluation of the adhesion and inactivation of E. coli on Ag-NPs/TiO_2-NTs under light and dark conditions

The antibacterial activity of the Ag-NPs/TiO_2-NTs was performed taking *Escherichia coli* (*E. coli* K12 ATCC 23716; from the Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (DSMZ), Braunschweig, Germany) as a probe. The samples were sterilized by autoclaving at 121 °C for 2 h. The 100 µL culture aliquots with an initial concentration of $\sim 4\ 10^6$ Colony-forming unit per milliliter (CFU mL^{-1}) in NaCl/KCl (pH 7) were placed on the samples. The 100 µL of the *E. coli* inoculum was contacted with the Ag-NPs/TiO_2-NTs uniform surface. The exposition was done at 23 °C (+/−2°). The samples were then placed on Petri dishes provided with a lid to prevent evaporation. After each determination, the surface was transferred into a sterile 2 mL Eppendorf tube containing 900 µL autoclaved NaCl/KCl saline solution. This solution was subsequently mixed thoroughly using a Vortex for 2 min. Serial dilutions were made in NaCl/KCl solution. A 100 µL sample of each dilution was pipetted onto a nutrient agar plate and then spread over the surface of the plate using standard plating method. Agar plates were incubated lid down, at 37 °C for 24 h before colonies were counted. Three independent assays were done for each sample.

The statistical analysis of the results were performed for the CFU values calculating the standard deviation values. The average values were compared by one-way analysis of variance and with the value of statistical significance. The one-way analysis of variance (one-way ANOVA) was used to compare the mean of the samples using the Fisher distribution. The response variable was approximated for the sample data obtained from the photocatalytic inactivation of test samples presenting the same distribution within the same sample (prepared at fixed anodization voltage). To verify that no attached/adsorbed bacteria remained on the surface, samples were incubated for 24 h at 37 °C on agar. No bacterial re-growth was observed.

The samples were irradiated in the cavity of an Atlas solar simulator (Atlas, GmbH, Hanau, Germany) with an overall power of 50 mW/cm^2 with light distribution wavelength distribution resembling solar irradiation emitting at wavelengths between 310 and 800 nm. The system contains an air-cooled Xenon lamp provided with filter to cut off wavelengths below 310 nm and above 800 nm. Samples were irradiated into covered glass petri dishes to avoid bacterial suspension evaporation due to the air-cooling. The material of the petri dishes does not cut the used light.

The adhesion of the *E. coli* on the TiO_2-NTs and Ag/TiO_2-NTs anodized surfaces was carried out by immersing the samples into 5 mL suspension of *E. coli* cell of a concentration of $4\ 10^6$ CFU/ml. The tube was then shacked gently at 37 °C for 4 h in dark [22,23]. The non-adhered bacteria to the NTs were removed by washing the surface with phosphate buffer solution (pH 7.2). The number of viable cells was determined after detachment of the adhered *E. coli* cells by ultra-sonication (50 W) for 15 min. Non-adhered/weakly adhered bacteria on the surfaces were evaluated according to Hoffman [24]. Bacterial adhesion experiments were carried out in the dark to avoid the photocatalytic action of the TiO_2 and Ag/TiO_2 surfaces.

2.3. Characterizations

The crystallinity and phase identification of the prepared samples were systematically investigated using X-ray diffraction (Philips X'PERT MPD, Cu Kα irradiation, λ = 1.5406 Å). The diffraction data were collected over the diffraction angle range of 20°–80° with a scanning step of 0.016°. The morphology and nanostructure of the Ag-NPs/TiO_2-NTs samples were examined by scanning electronic microscope (SEM, Jeol JSM-6300) and FEI Tecnai *G*2 transmission electron microscopy (TEM) operating at 200 kV with a LaB6 filament. The local chemical analysis was performed using the energy dispersive X-ray spectroscopy (EDXS) system attached to the TEM. The photoluminescence (PL) measurements on the Ag-NPs/TiO_2-NTs samples were carried out by using a fluorescence spectrophotometer (Perkin Elmer LS55) equipped with a xenon lamp at an excitation wavelength of λ = 340 nm. The XPS measurements were performed using an AXIS NOVA (Kratos Analytical, Manchester, UK) photoelectron spectrometer with an achromatic Al Kα X-ray source at 400 W. The spectra were excited using Al Kα radiation (1486.6 eV). All XPS data were corrected from sample charging during XPS data acquisition, while using the C1 s (284.6 eV) peak as a reference. The base pressure below $5\ 10^{-9}$ mbar was maintained during the measurement. No argon sputtering was considered as to clean the coatings surface, neither ion or electron neutralizers were used during the measurement. The surface atomic concentration percentage for each element was determined from peak areas using the known sensitivity factors for each element [25,26] on the surfaces. The spectrum background was subtracted according to Shirley [27] through the Shirley subtraction GL(30) program of the Kratos unit. The XPS spectra for the Ti and Ag-species were analyzed using CasaX-Vision 2 (Kratos Analytical, UK) and the peaks were assigned according to the NIST database. All measurements were done in the fixed analyzer