FeOx magnetization enhancing *E. coli* inactivation by orders of magnitude on Ag-TiO₂ nanotubes under sunlight.

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ABSTRACT

Drastic bacterial enhancement was observed when the Ag(3%)-TiO₂ nanotubes were modified with FeOx (3%) magnetic oxide. On bare TiO₂- nanotubes a reduction of 0.2log₁₀CFU was observed within one hour under simulated low intensity solar light. Under similar conditions, a bacterial reduction of 2.5log₁₀CFU was observed on Ag(3%)TiO₂ increasing to 6.0log₁₀CFU on Ag(3%)-TiO₂-FeOx(3%) magnetic nanotubes. The bacterial inactivation kinetics is strongly influenced by the addition of FeOx. The fast inactivation induced by the composite catalyst seems to involve an increase in the interfacial charge transfer (IFCT) compared to a 2-oxide composite photocatalyst. Stable recycling of the photocatalyst was observed leading to bacterial oxidation. The unambiguous identification of the radical intermediates: OH[•]-radicals, O-singlet and the valence holes vb(h+) on the Ag-TiO₂-FeOx interface showed that the valence band holes vb(h+) were the main oxidative intermediates leading to bacterial inactivation. Nanotubes size, crystallinity and bulk composition of magnetite 1% ($\theta = 51.0^\circ$), anatase 5% ($\theta = 8.9^\circ$), goethite 37.3 % ($\theta = 9.0^\circ$), silver 1% ($\theta = 2.7^\circ$) was obtained by the Rietveld refinement for the Ag(3%)-TiO₂-FeOx(3%) nanotubes. The redox chemistry during bacterial inactivation was determined by X-ray photoelectron spectroscopy (XPS).

KEYWORDS: Ag-TiO₂-FeOx synthesis, *E. coli* inactivation kinetics, mechanism, XRD, radical-scavenging.

1. Introduction

TiO₂ has been reported to absorb about 4% of the solar irradiation. A considerable effort has been undertaken during the last few decades to enhance their optical absorption into the visible range by doping, decorating the TiO₂ surface with metals, cations, anions, preparing binary-TiO₂ oxides to enhance the electron-hole separation/reaction kinetics [1-4]. An emerging field of interest is the synthesis of TiO₂ nanotubes and the coupling these nanotubes with cations, metal-oxides and additional composites leading to a higher nanotube sensitization in the visible range [5-7]. TiO₂ nanotubes (TNT) synthesis, dynamics and properties have been addressed due to their potential as photocatalysts [6-8] and incipient use in diverse type of photocells [9-10].

Antibacterial nanostructured titania nanotubes coating incorporated with silver nanoparticles have been reported. They have applications in Ti-implants to avoid post-operation infections [11-13]. The discoloration of the textile dye methyl red under UV-light and the degradation of pollutants by Ag-TiO₂ nanotubes has been recently reported [14-15]. Ag-TiO₂ was observed to accelerate bacterial inactivation when compared with bare TiO₂ under band-gap irradiation [16-17]. To increase the visible absorption of nanotubes, Fe-doping was carried out and their preparation and properties have been reported as well as their use in processes mediating dye degradation [18-19]. Fe-TiO₂ photocatalysts have also been reported recently to be effective in bacterial inactivation under solar irradiation [20-21].

The use of magnetic Ag-TiO₂-Fe₃O₄ composites to degrade chlorophenols has been reported [22]. The degradation of methyl red within 4 hours and bacteria within 2 hours on Fe-oxide added was reported but adding a much higher amount of Fe compared to the Fe used in the present study [23]. Also Fe₃O₄@TiO₂ core shell nanoparticle epoxide addressing the inactivating *E. coli* under solar light have recently been reported [24]. Iron oxide particles can be manipulated using a magnetic field gradient and have been investigated for their potential use in catalytic degradation of pollutants during water treatment [1-2], in biomedicine and in the biomedical fields [3-5].

We report hereby the synthesis of TiO₂ nanotubes modified by Ag and by magnetic FeOx leading to Ag-TiO₂-FeOx nanotubes. The objective is to show the acceleration of the disinfection kinetics by innovative magnetic nanotubes. This approach avoids the ultrafiltration or centrifugation necessary to separate the spent photocatalyst after the disinfection process. During the synthesis of the Ag-TiO₂-FeOx nanotubes Ag was

incorporated into the anatase by exchange followed by calcination. Fe-salts were then deposited on the Ag-TiO₂ nanotubes to add the magnetic properties. This enables the separation of the nanotubes from the solution by magnetic harvesting. A comprehensive, detailed and systematic study is reported in this work on the disinfection kinetics mediated by these novel nanotubes reporting on: the preparation of novel magnetic nanotubes under low intensity sunlight, the semiconductor intervention of the nanotubes in chemical transformations, the mechanism and kinetics of nanotubes during bacterial inactivation and the surface characterization of these innovative nanotubes.

2. Experimental

2.1 Ag-TiO₂-FeOx nanotubes hydrothermal synthesis.

The TiO₂ synthesis followed a modified the reported by Kasuga et al., [25] using TiO₂ P25 Degussa Aeroxide (Frankfurt, Germany). Kasuga et al., reported anatase nanotubes with a surface area of 50m²/g and presented TEM images of the TiO₂ nanotubes with a diameter of ca. 8 nm and a length ca. 100 nm. To ensure the removal of excess NaCl, the TiO₂ was washed with hot deionized water at 80°C. Addition of Ag was carried out and the Ag⁺ was subsequently reduced with 0.05M NaBH₄ in a cold bath at 5°C. The change of color from white to yellowish-brown indicates the chemical reduction of Ag. The resulting powders were calcined at 500° C for 1 hour, programming an initial at a heating rate of 250° C/hour. The Fe-incorporation on the Ag-TiO₂ nanotubes was carried out by co-precipitation adding the appropriate amounts of (NH₄)₂Fe(SO₄)₂·6H₂O (Sigma Aldrich) and NH₄Fe(SO₄)₂·12H₂O (Sigma Aldrich) in the molar ratio [Fe²⁺] : [Fe³⁺] of 1:1.5. After drying the powders, the products were harvested using a magnet and washed repeatedly with deionized water and ethanol. The powders were then dried at 70°C for 3 hours.

Table 1 presents the percentage of the Ti, Fe and Ag elements determined by X-ray-fluorescence (XRF) in the Ag-TiO₂ and Ag-TiO₂-FeOx nanotubes prepared in this study. The notation Ag(3%)-TiO₂-FeOx(3%) for the magnetic nanotubes corresponds to the make-up of the solutions used to prepare and load Ag and Fe to the preformed TiO₂ used as the TiO₂ source. The surface percentage of the Ti, Fe and Ag of the nanotubes throughout this study were determined by X-ray-fluorescence (XRF) and are reported in Table 1.

2.2. Evaluation of the *E. coli* inactivation kinetics during disinfection and irradiation procedures

Samples of *Escherichia coli* K12 (*E. coli* K12) were obtained from the Deutsche Sammlung von Mikro-organismen and Zellkulturen GmbH. The Luria-Bertani (LB) broth was prepared in Millipore water and subsequently sterilized [26]. A colony was picked from the pre-cultures and inoculated in 5 mL of LB broth. The vessel was incubated at 37°C with shaking at a rate of 180 rpm for 8 hours. Further dilution was made to a 1% v/v concentration and the solution incubated for 15 hours. The *E. coli* were harvested by centrifugation for 15 minutes at 4°C. The collected pellets were washed twice and re-suspended and sterilized. Aliquots with a bacterial concentration of 10^6 CFU/mL were generally used to follow the disinfection by the magnetic nanotubes irradiating in the cavity of a solar simulator (Atlas GmbH, Hanau, Germany) equipped with a cut-off filter < 310 nm and allow a variation of the light intensity between 25.2 mW/cm² and 46.5 mW/cm². The spectral emission of the solar simulator is shown in the Supplementary material S1. The samples to evaluate the bacteria CFU/ml were taken every 20 minutes by using one milliliter of the aliquots and transferring them to the appropriate Eppendorf tubes for further centrifugation. The samples were then spread-plated on agar (PCA) added to the sterilized Petri dishes. The plates were then incubated for 24 hours at 37°C and the results reported from triplicate bacterial runs.

2.3. Scavenging of the reactive oxygen species (ROS) and analysis of the leached Fe and Ag-ions

To evaluate the role of different ROS, scavengers were employed to identify ^1O by the azide NaN_3 , Ethylenediamine tetra-acetic acid (EDTA-2Na) to identify $\text{O}_2^{\cdot-}$ and Dimethyl sulfoxide (DMSO) to identify the $\text{OH}^\bullet$ -radical. The quantification of the ROS (mainly $\text{OH}^\bullet$) was carried out based on the method proposed by Hashimoto et al., [27]. The 99% terephthalic acid from Across Chemical Ltd, and the NaOH 98% were Sigma Aldrich and used as received. The photocatalyst was immersed in a 0.4 mmol L^{-1} solution of terephthalic acid dissolved in a 4 mmol L^{-1} NaOH solution. After irradiation, the solution was transferred to a quartz cell. The fluorescence of the 2-hydroxyterephthalic-acid was quantitatively monitored on a Perkin Elmer LS-50B spectrometer. The spectra were recorded between 400-500 nm (scan rate: 100 nm/ min) after excitation at 315 nm.

To determine the amount of the Fe-ions (II) and Fe(III) leached in solution the absorbance of Fe(III) was determined by a Ferrozine solution in acetate buffer solution at

562 nm in a Shimadzu UV-1800 spectrophotometer. Then, hydroxylamine hydrochloride (10% w/w) was added to the solution to reduce Fe^{3+} to Fe^{2+} and again the absorbance was measured again but this time of Fe^{2+} after 20 min at 562 nm. Ag-ions estimations in the ppb range were obtained by a Mohr titration to form AgCl that in the presence of chromate ion formed red-brown Ag_2CrO_4 .

2.4. X-Ray fluorescence, X-ray diffraction (XRD), X-ray photoelectron spectroscopy (XPS) and magnetic measurements

The X-ray fluorescence (XRF) of Ti, Fe and Ag was evaluated in an Analytical PW2400 spectrometer. The results are shown below in Table 1. The x-rays emitted by the atoms in the sample are collected by a detector, and processed in the analyzer to generate a spectrum showing the x-rays intensity peaks versus their energy. The peak energy identifies the element and its peak area (or intensity) gives an indication of its amount in the sample. The analyzer then uses this information to calculate the sample's elemental composition.

The evolution of powder crystallinity was investigated using X-ray diffraction (XRD) and recorded on an X'Pert MPD PRO from PANalytical equipped with a secondary graphite (002) monochromator and an X'Celerator detector operated in Bragg-Brentano geometry.

The magnetic measurements were performed with a Quantum Design NPMS-XI 5T Superconducting Interference Device (SQUID) magnetometer. The magnetic powder samples were mixed with a diluted GE-varnish and then measured at 300K in a field-sweep sequence $0\text{T} \rightarrow 5\text{T} \rightarrow -5\text{T} \rightarrow 5\text{T}$.

The X-ray photoelectron spectroscopy (XPS) of the nanotubes was determined using an AXIS NOVA photoelectron spectrometer (Kratos Analytical, Manchester, UK) provided for with monochromatic AlK_α ($h\nu=1486.6\text{ eV}$) anode. The carbon C1s line with position at 284.6 eV was used as a reference to correct the charging effects. The surface atomic concentration was determined from peak areas using the known sensitivity factors for each element [28-29]. Spectrum background was subtracted according to Shirley [30] and [52-53,60]. The XPS spectral peaks were deconvoluted with a CasaXPS-Vision 2, Kratos Analytical UK.

3. Results and Discussion

3.1. Kinetics of *E. coli* inactivation upon magnetization of the Ag(3%)-TiO₂ nanotubes and catalyst recycling.

Figure 1 shows the drastic increase in the bacterial degradation kinetics when Ag(3%)-TiO₂-FeOx(3%) nanotubes are irradiated under low intensity sunlight. Within an hour complete bacterial inactivation was attained that is a reduction of 6log₁₀CFU or full bacterial inactivation. During bacterial disinfection, the pH changes from ~6.8 to ~6.1. This pH change is important when discussing the Ag(3%)-TiO₂-FeOx(3%) mechanism leading to bacterial inactivation below in section 3.4. Ag- and Fe-cations ions intercalate on the TiO₂ lattice of the hydrothermally synthesized nanotubes [31-32] leading a synergistic effect in the Ag(3%)-TiO₂-FeOx(3%) accelerating the bacterial inactivation as shown in Figure 1, traces e). Figure 1, shows a very small bacterial reduction of 0.4log₁₀CFU in the absence of nanotubes due to the bacteria absorbing the incoming solar radiation below 340nm. The Ag(3%)-TiO₂-FeOx(3%) in the dark reduced the bacteria by 1.5log₁₀CFU within 80 min.

Chick-Watson (first order) kinetics ($\log(C/C_0) = k_1 \cdot t$) and Hom's equation ($\log(C/C_0) = -k_1 \cdot [1 - \exp(-k_2 \cdot t)]^{k_3}$) describe the trend shown for the bacterial inactivation kinetics in Figure 1. Three different kinetic stages adequately fit the Hom's equation with the parameters: k_1 = bacteria transformation to damaged species, k_2 = damaged bacteria transformation to inactivated species and k_3 = inactivated bacteria transformation to residual products. For the sample Ag(3%)-TiO₂-FeOx(3%), k_1 , k_2 and k_3 were 14.5, 0.02 and 4,0 respectively with an $R^2=0.9741$.

Several studies on the activity of Ag-TiO₂ enhancing the photocatalytic transformations of chemicals compared to TiO₂ have been reported [31-36]. Moreover some recent work describes the enhanced microbial inactivation of Ag-TiO₂ composites [37-38]. But the bacterial inactivation of Ag-TiO₂ compared to TiO₂ is strongly dependent on many variables that are difficult to control. During the photocatalysis, the Ag-ions which are the antibacterial agents on the Ag-TiO₂ surface, get reduced on TiO₂ to Ag⁰, a non-active antibacterial species [39] due to the cbe- of TiO₂.

Figure 2 indicates that some Fe(II)(III)-ions were released in the presence of *E. coli*, but the Fe-ions generation was not observed in the absence of bacteria. The kinetics of *E. coli* was also investigated as a function of the amount of Ag in the Ag-TiO₂-FeOx catalyst. Ag(3%) loading led to the fastest inactivation (see Supplementary Figure S2). A

100mg powder Ag(3%)-TiO₂-FeOx(3%) catalyst powder led to the fastest bacterial inactivation compared to other amounts of catalysts (see Supplementary Figure S3).

Under the experimental conditions applied in Figure 1 an initial load of 10⁶ CFU/ml is inactivated within 60 min. But this was not the case for an initial CFU of 10⁸/ml load (see supplementary Figure S4). This shows the limiting capacity of the active sites on the Ag(3%)-TiO₂-FeOx(3%) to abate bacteria. Figure 2 shows that the amount of Fe-ions released was far below the cytotoxicity levels for Fe(II)Fe(III) ions [40-41]. The small dissolution of the nanotube surface in the presence of bacteria has been reported due to the presence of Fe³⁺-siderophores presenting a big affinity for Fe and ability to chelate Fe-ions [42-43]. The oxygenated solution in Figure 2 shows a much higher level of Fe³⁺-ions compared to Fe²⁺-ions since the transformation of Fe²⁺ → Fe³⁺ k_1 76 M⁻¹s⁻¹ is much higher than the reverse reaction Fe³⁺ → Fe²⁺ 2x10⁻² M⁻¹s⁻¹ [1-2, 36]. The kinetics of *E. coli* was also investigated as a function of the amount of Ag in the Ag-TiO₂-FeOx catalyst. A catalyst loading of Ag(3%) loading led to the fastest inactivation (see Supplementary Figure S2).

Figure 3 shows the recycling of the Ag(3%)-TiO₂-FeOx(3%) nanotube powders up to the 5th cycle. No loss of activity was observed during the repetitive degradation an initial 10⁶ CFU bacterial concentration. The Fe leaching from the catalyst surface in the ppb range as shown in Figure 2, did not affect the bacterial inactivation kinetics up to the 5th cycle as shown in Figure 3.

3.2 Scavenging of the intermediate oxidative species leading to bacterial inactivation.

The contribution of ROS-species and of the valence band holes vb(h⁺) during the bacterial inactivation is presented next in Figure 4a. Figure 4a presents the scavenging of the radicals during bacterial inactivation under solar light irradiation up to 80 min [1-3, 43]. Figure 4a, trace a) presents the bacterial inactivation, no scavengers added within 60 min, b) trace b) presents the incomplete bacterial inactivation after the addition of 0.2 mM of NaN₃ an O-singlet quencher [44-45]. This compound inactivates bacteria by itself but not at the low concentration of 0.2 mM used during this study [46], c) trace c) shows further inhibition of the bacterial reduction upon addition of 0.2 mM methanol an OH[•]-radical scavenger and d) trace d) presents the almost initial inhibition of bacterial inactivation upon addition of 0.2 mM EDTA-2Na a well-known vb(h⁺) scavenger. Benzoquinone a quencher of O₂^{•-}/HO₂[•] was not assayed since even at very low concentrations it has been reported to inactivate bacteria by itself [1, 36, 46]. The relative

importance of the intermediate radical species leading to bacteria inactivation shown in Figure 4a is $vbh^+ > OH^\bullet > O\text{-singlet}$.

Reactive oxygen species (ROS) are chemically reactive molecules containing oxygen, like superoxide, hydroxyl radical, and singlet oxygen have been widely documented when semiconductor oxides like by TiO_2 , Ag_2O and $FeOx$ (mainly Fe_2O_3) undergo band-gap irradiation [1-5, 47-48]. Figure 4b shows the quantitative monitoring for the increase in $OH^\bullet$ -radical as a function of the PE- TiO_2 irradiation time. The increase in the fluorescence intensity is proportional to the $OH^\bullet$ -radical concentration in solution. Details of the analysis were described in the experimental section [27]. The increase of the fluorescence peaks at 450 nm is shown in Supplementary Figure S5. The data in Figure 4b confirms the presence of $OH^\bullet$ -radicals during bacterial disinfection as already reported in a qualitatively way in Figure 4a.

3.3. *Ag(3%)- TiO_2 -FeOx(3%) nanotubes behaving as semiconductors during to bacterial inactivation*

Figure 5 shows the effect of the applied light dose on the bacterial degradation kinetics. The shorter disinfection kinetics shown in Figure 5 as a function of a higher light dose providing an increasing amount of photo-generated charges is the evidence for the semiconductor behavior of $Ag(3\%)-TiO_2-FeOx(3\%)$. In a second step the semiconductor surface charges led to the ROS accounted for in Figure 4a. No saturation effects for the incoming radiation were observed in the $FeOx-TiO_2-PE$ film since the bacterial inactivation kinetics became shorter as the light intensity was increased.

3.4. *Mechanism suggested for the bacterial inactivation on $Ag(3\%)-TiO_2-FeOx(3\%)$ mediated by a two cascade process*

Figure 6 shows that in the coupled semiconductors the $cb(e^-)$ injection and the $vb(h^+)$ are a function of their relative potential energy levels. Since the bacterial degradation kinetics was faster on $Ag(3\%)-TiO_2-FeOx(3\%)$ (Figure 1, trace e) compared to the one induced by $Ag(3\%)-TiO_2$ (Figure 1, trace c) this shows that that in the presence of $FeOx$, the additional charge transfer states accelerate the charge transfer from the semiconductors to bacteria.

The mechanism shown in Figure 6 involves sunlight radiation reaching $AgOH$. But $AgOH$ decomposes spontaneously to Ag_2O (eq.1) [4-50]

This Ag₂O has been reported to be thermodynamically stable region at pH 6-7, the pH domain of bacterial inactivation. Sunlight irradiation photo-activates Ag₂O with a bg ~1.5 eV [43] as noted in eq.(2):

The transfer of the Ag₂Ocb (e-) to FeOx (mainly goethite FeOOH 37.3%) precludes electron-hole recombination in the Ag₂O and leads to the formation of the highly oxidative radicals inactivating bacteria as shown in the upper right hand side in Figure 6. Direct and indirect band gaps for nano-goethite with particle sizes as shown in Table 1 have been reported [50]. The Ag₂Ocb (e-) reacts with O₂ (air): e- + O₂ → O₂⁻ giving rise to the radical chain shown in Figure 6 and concomitantly the Ag₂Ovb(h+) reacts: h⁺ + H₂O → OH^o + H⁺. The local pH of the Ag(3%)-TiO₂-FeOx(3%) film was followed during bacterial inactivation and decreased from 6.8 to 6.1. This means that H⁺ generation predominates over the generation of OH- displacing the pH in the solution to more acidic values.

The electron transfer from goethite (FeOOH) proceeds to the lower lying anatase electron trapping states located about 0.8-1.0 eV below the TiO₂cb [51] since goethite (cb) is positioned ~0.5-0.8 eV below TiO₂cb [52]. The FeOOH photo-generated charges being very short lived due to its small band gap would increase their lifetime due to cbe- transfer to the anatase (trapping states) or the concomitant vbh+ injection into Ag₂O [48] In Figure 6 the goethite-TiO₂ interfacial charge transfer (IFCT) is suggested to involve the following steps:

An alternative shorthand mechanism is suggested below in the overall eq.(9)

3.5. X-Ray diffraction (XRD), particle size, magnetic properties and visual perception of samples.,

Figure 7a shows the XRD-spectra for Ag (1%, 3%, 5%)-TiO₂ nanotube powder with the TiO₂ peaks and Ag-peaks. The XRD became broader upon an increasing Ag-concentration as reported by Toledo et al., [53] and Lei et al., [54]. Table 1 summarizes the size of TiO₂ anatase crystallites, calculated from the peak fitting of the (101) reflection in the XRD spectrogram by way of the Debye-Scherrer noted below in eq.(10)

$$D = \frac{k\lambda}{\beta \cos\theta} \quad (\text{Eq.10})$$

Where, D = crystallite size (nm), k = shape factor (usually taken as 0.89), λ = X-ray wavelength (taken as 0.15406 nm), β = FWHM (radians), θ = Bragg's diffraction angle (radians).

The presence of Ag⁰ in the Ag-TiO₂ samples was detected by XRD along additional peaks commonly ascribed to Ag₂O. Table 2 shows that Ag reduces the crystallite size of anatase in agreement with a study recently reported [46]. In this reference, the Ag-TiO₂ was prepared starting from butyl titanate as titanium source, adding the desired amount AgNO₃ and after a sol-gel drying the gel at 60°C, calcining the TiO₂, Ag-TiO₂ at 400°C and 500°C. By using Scherrer's equation, the size of TiO₂ samples was found to be 34 nm, decreasing to 12 nm after heating at at 400 °C and to 10.9 nm after heating at 500 °C. The drastic reduction of the TiO₂ crystallite size upon addition of Ag was explained to the effect of Ag inhibiting in the TiO₂ gel grain growth. In the present study we have used pre-formed TiO₂ P25 Degussa Aeroxide (Frankfurt, Germany) adding Ag as described in the experimental section 2.1 and subsequently calcining at 500 °C for 1 hour. The reduction in the particle size upon Ag-doping was estimated by the Scherrer's eq(10) and the results shown in Table 2. Upon addition of Ag s the sample size shows a reduction of the TiO₂ size from 17 to 12 nm for an Ag(5%) doped sample. The reduction in the crystallite sizes is ascribed to an increase in the TiO₂ crystallite compactness as the amount of Ag in contact with the TiO₂ sample increases. Samples were calcined for the same time (1 hour) at 500°C. In the supplementary Figures S6, the XRD of TiO₂ P25 Degussa is shown along the XRD of the nanotubes heated at 500 °C. A close resemblance was observed due to the presence of anatase and an additional phase with a monoclinic symmetry. The complete transformation for H₂Ti₃O₇ to titanate at 500°C was reported by Tsai and Teng] reporting atomic plane d-spaces of 0.58 [54].

Upon incorporation of the magnetic component FeO_x , the XRD of the $\text{Ag}(3\%)\text{-TiO}_2\text{-FeO}_x(3\%)$ nanotube changes considerably and this is seen by inspection of Figure 7b. The composition of this sample as determined by the Rietveld refinement were: magnetite 1% ($\theta = 51.0^\circ$), anatase 5% ($\theta = 8.9^\circ$), goethite 37.3 % ($\theta = 9.0^\circ$), silver 1% ($\theta = 2.7^\circ$) and the rest was rutile and a more significant percentage of TiO_2 amorphous. The Rietveld refinement implies material characterization by neutron and X-ray diffraction to obtain the reflections (peaks in intensity) at certain positions [55]. A peak reduction in the anatase reflections of (101) and (020) was observed on the magnetite and goethite peaks. It has been reported by Selmani et al., [56] and Grover et al., [57] that the magnetite/goethite incorporation in a nanocomposite was a function of: the applied temperature, the co-precipitation pH in the aqueous solution and the sample exposition to N_2 or air during heat treatment /calcination. Using eq.(10) in the $\text{Ag-TiO}_2\text{-FeO}_x$ nanocomposite, the estimated crystallite sizes obtained using the reflections of (311) for magnetite and (110) for goethite were 7.8 nm and 5.1 nm respectively. The magnetization of the samples is shown in in Figure S7 of the Supplementary material. In the supplemental material the samples S7a $\text{TiO}_2\text{-FeO}_x(0.5\%)$; S7b $\text{Ag}(1\%)\text{-TiO}_2\text{-FeO}_x(3\%)$; S7c $\text{Ag}(3\%)\text{-TiO}_2\text{-FeO}_x(3\%)$ and S7d $\text{Ag}(5\%)\text{-TiO}_2\text{-FeO}_x(3\%)$ present magnetic properties. The sample S7e $\text{TiO}_2\text{-FeO}_x(1\%)$ is paramagnetic showing saturation behavior. The magnetization level detected in the sample S7e was about a hundred times smaller compared to the samples S7b, S7c and S7d.

The isoelectric point (IEP) of goethite has been reported with values 7.5-8.5. At a pH 6-7 used during the bacterial inactivation, the surface Fe-OH_2^+ interacts electrostatically with bacteria [58]. Nano-goethite with a band-gap of 2.2 eV, under sunlight irradiation generates ROS as shown in Figure 4a. Goethite being a weak magnetic material will decrease the magnetization of magnetite (band gap of 0.1 eV) [57].

The significant difference in the visible perception of TiO_2 , Ag-TiO_2 and $\text{Ag}(3\%)\text{-TiO}_2\text{-FeO}_x(3\%)$ nanotube powders is seen in Figure 9a/9b. The dark coloring effect by FeO_x is readily seen in Figure 9b.

3.6. Surface composition and redox shifts detected by XPS during bacterial inactivation

Figure 8a(a) shows the initial values (time zero) for the binding energies of the Ti found in the XPS spectrogram of the $\text{Ag}(3\%)\text{-TiO}_2\text{-FeO}_x(3\%)$ nanotubes. The peak positions reported in Figure 8 were consistent with the values reported in references [28-29]. Figure 8a(b) shows the corresponding XPS peaks after one bacterial inactivation. A

shift of 0.4 eV for TiO₂ and TiOH was observed after bacterial inactivation (Figure 8a(b)). Shifts ≥ 0.2 eV imply the appearance in the XPS spectrogram of new species on the catalysts surface [30,58]. The interaction of the bacteria with the photocatalyst surface comprises the electrostatic interaction of the positively charged TiO₂, Fe₂O₃ and Ag₂O oxides with the negative charged *E. coli*.

Figures 8b(a) and 8b(b) report the shifts of the AgOH peak from 366.8 eV to 366.0 eV. The Ag₂O peak remained stable before and after bacterial inactivation showing only a shift of ~ 0.1 eV. The small difference in the relaxation energies for Ag(I) and (Ag II) is responsible for their very close BE-energies [59,60] and the assignment of these peaks is complicated by the electrostatic charging for Ag-oxides. Figure 8c(b) presents XPS-peak shifts after one hour with respect to Figure 8c(a) at zero time comprising: a) a small shift for FeOOH of 0.2 eV, b) a stronger shift for the magnetic species Fe₃O₄ of 0.3 eV and c) a FeO peak shift of 0.2 eV. The observed XPS-shifts indicate that new species appear in the FeOx nanoparticles after bacterial inactivation.

4. Conclusions

In summary, a synergistic effect between Ag and Fe in Ag(3%)-TiO₂-FeO_x (3%) accelerate the bacterial reduction of *E. coli* under low intensity solar irradiation within 60 min. The kinetics of bacterial inactivation on Ag(3%)-TiO₂-FeO_x(3%) nanotubes was seen to proceed with a 3.5log₁₀CFU order of magnitude increase over the bacterial inactivation mediated by Ag(3%)-TiO₂ nanotubes. The effect of the variation of the applied solar dose suggests that the bacterial inactivation kinetics is a function of the photo-generated charges on the oxides making up the photocatalyst. The transients leading to bacterial inactivation such as: $h\nu^+$, HO₂⁻, and ¹O-singlet were unambiguously identified. The Fe-cation leached in ppb amounts during bacterial inactivation does not have a significant effect on the bacterial degradation kinetics due to its low concentration. A mechanism of reaction is suggested based on the potential energy position of the electronic bands making up the Ag(3%)-TiO₂-FeO_x(3%) nanotubes. The surface properties of the nanotubes were investigated by XRD and XPS. Bacterial inactivation proceeded without adding chemicals on photo-stable and reusable nanotubes. The nanotubes were magnetically recoverable by an external magnet for subsequent reuse.

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Table 1. Percentage of the Ti, Fe and Ag elements determined by X-ray-fluorescence (XRF) in the Ag-TiO₂ and Ag-TiO₂-FeOx nanotubes prepared in this study.

Sample	Ti	Fe	Ag
3% Ag-TiO ₂	85	None	12
1% Ag-TiO ₂ -FeOx	30.1	68.6	1.2
3% Ag-TiO ₂ -FeOx	22.7	72.8	3.3
5% Ag-TiO ₂ -FeOx	20.2	74.2	5.1

Table 2. Summary of average crystallite size calculated from the strongest peak signal.

Sample no. (% Ag)	2 θ (101)	β (FWHM)	D (nm)
Sample 2 (0%)	25.25	0.4856	17.01 nm
Sample 3 (1%)	25.28	0.5103	16.17 nm
Sample 4 (3%)	25.22	0.5825	14.13 nm
Sample 5 (5%)	25.25	0.6729	12.20 nm

Figure 1. Bacterial inactivation under low intensity sunlight irradiation (46.5 mW/cm²) and in the dark mediated by 100 mg nanotube powder in dispersions with different compositions: a) no catalyst control run, b) bare TiO₂ nanotube dispersions under light, c) Ag(3%)-TiO₂ under light, d) Ag(3%)-TiO₂-FeOx(3%) in the dark under and e) Ag(3%)-TiO₂-FeOx(3%) under light irradiation.

Figure 2. Determination of the Fe-ions production within the time in the presence of *E. coli* on Ag(3%)-TiO₂-FeOx(3%) nanotubes under solar simulated light (46.5 mW/cm²).

Figure 3. *E. coli* inactivation e cycles of 3%Ag-TiO₂-FeOx(3%) under low intensity solar simulated light (46.5 mW/cm²).

Figure 4a. Bacterial inactivation by Ag(3%)-TiO₂-FeOx(3%) composite nanotubes (100 mg) under solar simulated light (46.5 mW/cm²): a) no scavengers, b) NaN₃ (0.2mM) an O-singlet scavenger, c) DMSO (0.2mM) an OH[•]-radical scavenger, and d) EDTA-2Na (0.2mM) a vb(h⁺) scavenger.

Figure 4b. Evolution in time of the increase in fluorescence of a solution 2-hydroxyterephthalic acid irradiated up to 80 min. For additional details see Ms text.

Figure 5. Dependence of the bacterial inactivation kinetics on the solar simulated solar irradiation dose applied on a sample containing Ag(3%)-TiO₂-FeOx(3%) nanotubes.

Figure 6. Ag(3%)-TiO₂-FeOx(3%) interfacial charge transfer as a function of their relative potential energy levels.

Figure 7a. X-Ray diffraction of 1%, 3% and 5% Ag-TiO₂ prepared for nanotubes referenced to pure Ag and TiO₂ calcined anatase powders.

Figure 7b. X-Ray diffraction of Ag(3%)-TiO₂-FeOx(3%) nanotubes referenced to anatase, goethite, magnetite and silver powders.

Figure 8a. Ti2p deconvolution of the XPS peaks of Ag(3%)-TiO₂-Fe(3%) nanotubes: (a) before bacterial inactivation and (b) after bacterial inactivation within one hour under low intensity solar light (50 mW/cm²).

Figure 8b. Ag3d deconvolution of the XPS peaks for Ag(3%)-TiO₂-Fe(3%) nanotubes: (a) before bacterial inactivation and (b) after bacterial inactivation within one hour under low intensity solar simulated light (50 mW/cm²).

Figure 8c. Fe2p deconvolution in Ag(3%)-TiO₂-Fe(3%) nanotubes: (a) before bacterial inactivation and (b) after bacterial inactivation under low intensity within one hour under solar simulated light (50 mW/cm²).

Figure 9a. Powder samples of Ag(1%)-TiO₂, Ag(3%)-TiO₂ and Ag(5%)-TiO₂

Figure 9b. Powder samples of TiO₂, Ag(3%)-TiO₂ and Ag(5%)-TiO₂-Fe(3%)

Graphical abstract: