Photoinduced bactericidal activity against *Pseudomonas aeruginosa* by TiO_2 based thin films

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Abstract

The photoinduced bactericidal capacity of TiO_2 based films was evaluated, using as model organism Pseudomonas aeruginosa. Thin films were obtained by spray pyrolysis; they included undoped, Cu doped, and Al doped TiO_2. Scanning electron microscopy was used to observe the final effect of the irradiated films upon the bacteria. Depending on the composition and characteristics of the films, quantitative experiments show that bacterial inhibition varies between 28 and 96%. The order of magnitude of the average quantum yield of the films was determined between 10^{-9} and 10^{-11} inhibited bacterial per photon.

Keywords: Titanium dioxide, Photodegradation, Bacterial inhibition, Photocatalysis.

Introduction

Photocatalytic materials such as titanium dioxide (TiO_2) have been studied for more than 20 years on their capacity to degrade organic contaminants from air and water. Because of its unique photoinduced characteristics, it has been widely studied for applications such as auto cleaning agent, deodorant, antibacterial, and for puri¢cation of water and air [1,2]. For example, Matsunaga et al. [3] reported a signi¢cant reduction in the number of microbial cells suspended in an aqueous solution after 60^120 min contact with a layer of TiO_2-Pt exposed to UVradiat ion. The same authors constructed

a photochemical induced bacterial filter from a nitrocellulose membrane added with titanium dioxide [4]. Photoinduced bactericidal activity of titanium oxide can lead to di¡erent applications, including disinfections in diverse environments [5,6]. In the ceramic and building industry, the photoinduced bactericidal effect of titanium oxide can be of special interest. This is particularly true when the ceramic is going to be placed in microbiologically sensitive environments, such as medical facilities, production or experimental environments where biological contamination must be prevented.

In this work, we report the preparation and the bactericidal effect of TiO_2 based films, including undoped, Cu doped and Al doped TiO_2. The films were deposited on soda lime glass slides and inside fused silica tubing by spray pyrolysis. There are numerous works dealing with the bactericidal effect of a TiO_2 photocatalyst over a wide range of microorganisms [3-7]. However, in our knowledge, this work is the first report on an estimation of the order of magnitude of the average quantum efficiency of the TiO_2 based films, which is a better indicator of their bactericidal capacity. In addition, also presented for the first time is an evaluation of the bactericidal effect on *Pseudomonas aeruginosa.*

Materials and methods

Bactericidal inhibition and average quantum yield: Usually bactericidal activity is evaluated by the percentage of bacterial inhibition (<BI>)

$$\langle \mathrm{BI} \rangle = \frac{C_0 - C_F}{C_0}$$

where C_0 and C_F are the initial (before irradiation) and final (after irradiation) concentration of bacteria, respectively [3,5-7]. However, it is well established that knowledge of quantum efficiency of a photocatalytic process is fundamental: (a) to compare the activity of different catalysts for the same reaction, (b) to estimate the relative feasibility of di¡erent reactions, and (c) to calculate the energetic yield of the process [8]. For this reason, the average quantum efficiency (<QY>) of the films was estimated by the following arguments. First of all, QY is defined as the ratio of the number of events (in the present case bacterial death) taking part in the photoreaction per unit time to the number of absorbed photons per unit time [9] :

$$\mathrm{QY} = \frac{\left(\frac{\mathrm{d}N}{\mathrm{d}t}\right)}{\left(\frac{\mathrm{d}N_{\mathrm{ph}}}{\mathrm{d}t}\right)}$$

where, in the case of bacterial inhibition, dN/dt is the inhibition rate (death bacteria s^{-1}) and dN_{ph}/dt is the rate of photons absorbed by the film (photons s31). In general, the QY is a function of time, concentration of bacteria, light intensity, and wavelength (energy) of the photons. From this definition, the average quantum yield <QY> can be stated as follows:

$$\langle \mathrm{QY} \rangle = \frac{\frac{1}{\Delta t}\int_0^{\Delta t} \frac{\mathrm{d}N}{\mathrm{d}t}\mathrm{d}t}{\frac{1}{\Delta t}\int_0^{\Delta t} \frac{\mathrm{d}N_{\mathrm{ph}}}{\mathrm{d}t}\mathrm{d}t} = \frac{\Delta N}{\langle N_{\mathrm{ph}} \rangle}$$

Where ΔN is the total number of inhibition bacteria, $<N_{ph}>$ is the total number of absorbed photons (365nm), and Δt is the irradiation time. ΔN was calculated starting from:

$$\Delta N=(C_0-C_F).V$$

Where V is the inoculum's volume. Additionally, $<N_{ph}>$ was estimated from:

$$\langle N_{ph}(\lambda)\rangle = \frac{I_o(\lambda)\cdot S\cdot \Delta t}{\varepsilon(\lambda)}\alpha(\lambda)$$

Where $I_o(\lambda)$ is the irradiance (W cm^{-2}) at λ, ε(λ) is the photon energy (J) S is the effective film surface area irradiated (cm^{-2}) at λ, ε(λ) is the photon energy (J), S is the effective film surface area irradiated (cm^2), Δt is the irradiation time and α(λ9 is the absorbance of the film at λ. For the case of film covered glass slides, the absorbance can be expressed as:

$$\alpha(\lambda) = \tau_1(\lambda)\cdot(1-\tau_F(\lambda)-\rho_F(\lambda))$$

Where $T_1(\lambda)$ is the transmittance of the inoculum placed over the film; Tf (λ) and $\rho_F(\lambda)$ are the transmittance and reflectance of the film in the flass slides T_1 (λ) has been evaluated as:

$$\tau_1(\lambda) = e^{-a(\lambda)\cdot h}$$

Where a(λ) is the absorption coefficient of the inoculum and h is the inoculum height over the film. The coefficient a(λ) was determined experimentally from spectral transmittance measurement (A≈ 0.3 mm^{-1} for λ=365 nm). Furthermore, for the film covered tubing, α(λ) was evaluated considering:

$$\alpha(\lambda) = \tau_V(\lambda)\cdot(1-\tau_{FV}(\lambda)-\rho_{FV}(\lambda))$$

Where T_v (λ) is the transmittance of the fused silica tubing without films (T_v = 0.81) ; T_{FV} (λ) and ρ_{FV} (λ) are the transmittance and reflectance of the film in the fused silica tubing, respectively. The projection of the tubing surface on a plane normal to the direction of irradiation has been considered as the irradiation surface as well. The inoculum volume was considered to establish the height of the tubing surface that is involved in the inhibition process. Finally, the average energy yield (<EY>(was calculated from <QY>:

$$\langle EY \rangle = \frac{\varepsilon(\lambda)}{\langle QY \rangle}$$

Thin film preparation and characterization: A spray pyrolytic system was used to prepare TiO_2 based films on soda lime glass slides (40x25 mm^2). Details of the system are reported elsewhere [10]. The precursor solution was a dilution of titanyl acetyl acetonate in absolute ethanol; their concentration was kept at 0.1 mol dm^{-3}. Cupric acetate and aluminium acetyl acetonate were used as the dopant source for Cu and Al doped films, respectively. X-Ray diffraction (XRD) was employed to study the crystalline structure of the films deposited on glass plates. For doped films, the Cu/Ti and Al/Ti atomic ratios were determined by X-ray energy dispersive spectroscopy (EDS). Additionally, TiO_2 thin ¢lms were obtained inside fused silica tubing by a new, very simple spray pyrolysis technique; the details of the preparation method are reported elsewhere [11]. For these samples, transmission electron microscopy (TEM) and

selected area electron diffraction (SAED) analysis were used to evaluate the morphology and crystalline structure. Spectral transmittance and reflectance measurement in the UVVIS interval was also performed to determine thickness and optical properties of the films. Table 1 summarizes the optimized experimental conditions used in the preparation of the films, and relevant properties.

Quantitative inhibitory test: A wild-type P. aeruginosa strain was used in the inhibitory test (Facultad de Ciencias Químicas, UACh, Strain Collection). Bacteria were cultured on nutrient broth for 24 h at 37°C. A bacterial inoculum (0.15 cm^3), with known initial cell concentration (around 10^8-10^9 colony forming units (CFU) cm^{-3}), was placed on top of the film covered substrates and on a glass slide without any film as a positive control. To limit and fix the contact area between the inoculum and the film, a Teflon ring was firmly attached to the glasses; the ring also served to establish the height of the inoculum volume over the film's surface. The film covered substrates and control were placed inside an irradiation chamber, and exposed to a long-wave black light (365 nm) for 80 min. In the case of fused silica tubing, 2 cm^3 of the known inoculum were placed inside the film covered and non-covered tubing (one extreme sealed).

Table 1
Experimental conditions used in the preparation of both film covered glass slides and tubing

Film	Material	T_s (°C)	T_c (°C)	t (min)	f (cm^3 s^{-1})	d (nm)	v (nm s^{-1})	at. % dopant/Ti		Phase
								solution	film	XRD
Ti-824	TiO_2	450	350	30	67	356	0.20	–	–	Amorphous
Ti-826	TiO_2	450	400	30	67	396	0.22	–	–	Anatase
Ti-828	TiO_2	450	450	28	67	546	0.33	–	–	Anatase
Ti-908	TiO_2	450	450	30	133	746	0.41	–	–	Anatase
Cu doped TiO_2 films on glass substrates										XRD
CuTi-16	TiO_2:Cu	400	400	20	67	323	0.27	3.0	15	Amorphous
CuTi-201	TiO_2:Cu	400	400	30	67	455	0.25	0.75	4	Amorphous
Al doped TiO_2 film on glass substrates										XRD
TiAl-306	TiO_2:Al	450	450	33	133	330	0.17	0.8	5	Anatase
TiAl-916	TiO_2:Al	450	450	24	133	594	0.41	10	35	Amorphous
TiO_2 film inside vycor tubing										SAED
T-019	TiO_2		500	6.4	67	119	0.31	–	–	Anatase
T-023	TiO_2		500	12.5	50	205	0.27	–	–	Anatase
T-026	TiO_2		500	4.5	67	71	0.26	–	–	Anatase-Rutile
T-027	TiO_2		500	8.5	58	114	0.22	–	–	Anatase-Rutile

Material, type of film; T_s, substrate temperature; T_c, furnace temperature; t, deposition time; f, carrier gas flux; d, film thickness; v, deposition rate; at. % dopant/Ti, atomic percentage of dopant/Ti ratio; phase, crystalline phase of the films.

These tubings were stacked vertically and exposed to the same black light lamp for 40 min, every 10 min the tubings were rotated 180°. The irradiance of the lamp, at 365 nm, was measured by means of a UVP radiometer model UVX. After exposure, 0.1 and 0.5 cm^3 of the bacterial inoculum were recollected from the Teflon ring and tubing, respectively, to determine the final cell viability. Serial dilutions to a final concentration of 10^{-7} and spread plate techniques on plate count were used to determine bacterial cell concentration (CFU cm^{-3}) [12]. Scanning electron microscopy (SEM) was used to show unequivocally the effect of the irradiated film upon the bacteria.

Table 2
Results of quantitative bacterial inhibition test

TiO_2 film	C_0 ($\times 10^8$ CFU cm^{-3})	ΔN ($\times 10^7$ CFU)	⟨BI⟩ (%)	α (%)	I_o (μW cm^{-2})	S (cm^2)	$N_{ph.abs}$ ($\times 10^{17}$)	⟨QY⟩ ($\times 10^{-11}$)	⟨EY⟩ ($\times 10^{-8}$ J)
Non-covered	1.30	0.15	8	59[a]	469	0.50	12.3	0.12	44.6
Ti-824	1.30	1.26	65	20	469	0.50	4.10	3.08	1.77
Ti-826	1.30	1.19	61	25	469	0.50	5.29	2.24	2.43
Ti-908	1.30	0.98	50	16	469	0.50	3.24	3.01	1.81
CuTi-201	1.40	1.20	57	14	450	0.50	2.80	4.28	1.27
TiAl-916	1.80	7.50	28	15	450	3.80	22.5	3.33	1.64
TiAl-306	1.40	0.95	45	22	450	0.50	4.42	2.14	2.55
Vycor tubing									
Non-covered	11.6	32.0	14	74[a]	475	3.64	56.2	5.69	0.96
Ti 19	11.6	208.0	90	24	475	3.64	18.6	112	0.05
Ti 23	11.6	223.0	96	31	475	3.64	23.8	93.4	0.06
Ti 26	11.6	199.0	86	22	475	3.64	16.9	118	0.05
Ti 27	11.6	118.0	51	23	475	3.64	17.3	68.0	0.08

C_0, initial bacterium concentration; ΔN, total number of inhibited bacteria; ⟨BI⟩, bacterial inhibition; α, absorbance of the film at 365 nm; I_o, irradiation intensity peak at 365 nm; S, effective irradiated surface; $N_{ph.abs}$, total absorbed photon number; ⟨QY⟩, average quantum yield; ⟨EY⟩, average energy yield. Irradiation time of film covered glass slides and tubing was 80 and 40 min, respectively.
[a]In these cases it is considered the absorbance of the inoculum.

Results and discussion

Bactericidal activity: Table 2 shows the results of the bactericidal effect of the films determined by quantities as <BI> and <QY> ; these quantities have been calculated using the expression developed in Section 2.1. The irradiated non-covered glass plate had only 8% of <BI>. Nevertheless, for irradiated ¢lm covered plates <BI> varied between 28 and 65%. On the other hand, the irradiated non-covered tubing had only 14% of <BI>. Nevertheless, for irradiated TiO_2 based film covered tubing the <BI> ranged from 51 to 96%. Discussion of the influence of dopant type is presented in the light of average quantum efficiencies of the films. As mentioned before, the <QY> value is a better indicator of the bactericidal activity of the material than solely the percentage of bacterial inhibition (<BI>), because the former takes into account the number of inhibited bacteria and absorbed photons in the film. The <QY> for all film covered glass slides lies in the range of 10^{-11} inhibited bacteria per photon.

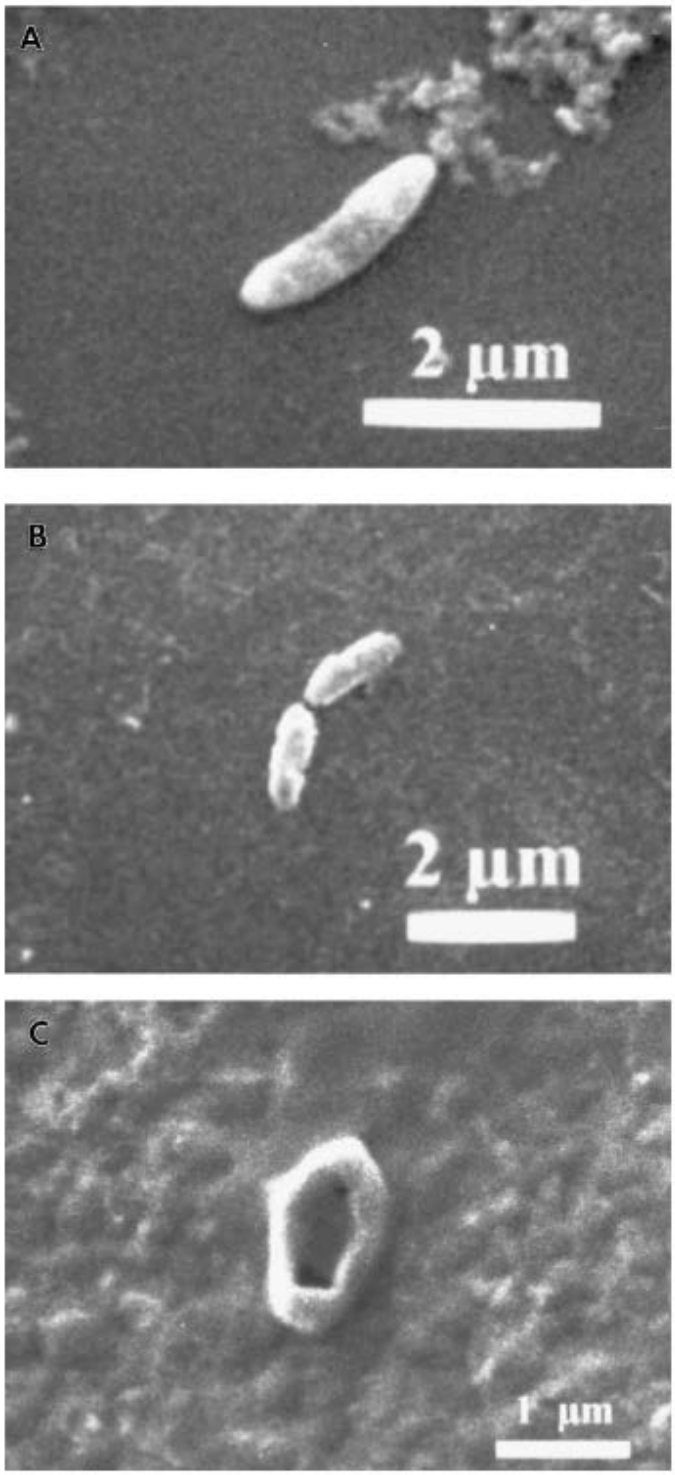

Fig. 1. Secondary electron SEM images of typical bacteria observed on the film surface. A: Bacteria on non-irradiated control glass slide; B: bacteria on irradiated control glass slide; C: irradiated bacteria on TiO_2 covered glass slide (Ti-824).

It is interesting to notice that, while the <BI> of film CuTi- 201 is slightly lower (57%) than that of Ti-824 (65%) and Ti-826 (61%), the <QY> of this film is considerably higher ($4.28x10^{-11}$) than that of Ti-824 ($3.08x10^{-11}$) and Ti-826 ($2.24x10^{-11}$). It means that the film CuTi-201 is more efficient than Ti-824 and Ti-826, as expected due to the presence of Cu. The <QY> of film covered tubing is higher than that of covered glass slides ; it is in the range from 10^{-10} to 10^{-9} inhibited bacteria per photon. It should be pointed out that our estimation of <QY> is affected by several factors (optical property uncertainties, irradiation intensity variation, short wave length influence, and biological aspects); however, the expected overall uncertainty is around a factor of two. In Table 2, there is also tabulated the average energy yield (<EY>) expressed as the average energy (J) necessary to inhibit the growth of one bacterium. The bactericidal activity reported here is consistent with observations previously reported by other authors for other bacteria [5-7, 13-15].

Direct observation of film effect by SEM: Fig. 1 shows secondary electron SEM images of typical bacteria observed in this work. Fig. 1A shows bacteria on the non-irradiated control glass slide; it can be seen that the bacteria's surface is smooth, indicating that no damage has occurred to the cell structure. The same conclusion can be deduced from the image of irradiated bacteria on the control glass slide (Fig. 1B). On the other hand, irradiated bacteria (80 min) deposited on the titanium dioxide covered glass slide (Ti-824) show a very rough surface or a hollow shape, suggesting that severe damage has occurred to the cell structure (see Fig. 1C). This micrograph supports the idea that cell death takes place by disruption of the cell wall, cell membrane and leakage of the cell contents [7].

Conclusion

Titanium dioxide based films obtained by spray pyrolysis have shown a photoinduced bactericidal effect on P. *aeruginosa*.

Quantitative experiments show that bacterial inhibition varies between 28 and 96%, depending on the composition and characteristics of the films. The <QY> for irradiated film covered glass slides lies in the range of 10^{-11} inhibited bacteria per photon, and that of the irradiated film covered tubing varies between 10^{-9} and 10^{-10}.

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