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Biocide Activity of TiO_2 Nanostructured Films

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Abstract. The ability of nanostructured TiO_2 in anatase phase to eliminate *Escherichia coli* (*E. coli*) by UV light irradiation was tested using titania films supported on glass substrates. The films were obtained by electrophoretic deposition of titania sol on sputtered Ti Corning glass substrates. Experimental procedure used to obtain these films and their characterizations are discussed in this paper. Nanostructure nature of the films was analyzed using scanning electron microscopy and atomic force microscopy. Optic microscopy was used to study the photocatalytic activity of films and their interaction with *E. coli* bacteria, in order to measure the reduction in *E. coli* colonies. The structure of anatase TiO_2 was determined using grazing incidence X-ray diffraction.

Introduction

The study of TiO_2 in anatase phase is important because of its potential to eliminate microorganisms [1] and its biocide activity [2] when it is irradiated with a specific wavelength of light that causes an electron to jump from valence band to conduction band. Gordillo Delgado *et al.* [3] prepared TiO_2 (titania) films by reactive sputtering, in order to add nitrogen to the surface of films. They studied the photocatalytic activity of TiO_2 films during irradiation with visible light, and demonstrated successful degradation of an organic compound, but did not test the degradation of bacteria. Several researchers have investigated the photocatalytic activity of TiO_2, and demonstrated that this photocatalyst can be used to treat pollutants and even to eliminate human pathogenic microorganisms [2, 3]. In this work, it has been hypothesized that if a titania thin film is formed by nanometric size particles, surface area will greatly increase, providing more reactive surface and thus enhancing the biocide activity [4-8].

Escherichia coli is a bacterium that is commonly found in the lower intestine of animals. Most *E. coli* strains are harmless, but some, such as serotype O157:H7 can cause serious problems in humans, even death. We were therefore interested in producing a nanostructured titania thin film with high biocide activity. Standard test to probe the biocide activity of such films requires growth of bacteria in a culture. A culture is the growing of microbial populations in a controlled way, in an artificial environment [8, 9]. The use of titania films as a biocide makes easy contact in the process avoiding the need for recovery of titania powders that arises when powders are used instead of films. In this study, a combination of methods was used to obtain TiO_2 films: sputtering and electrophoretic deposition [10-12]. In order to obtain a conductive substrate with similar composition to titanium oxide, sputtering [13] of metallic titanium on glass was proposed, followed by the use of these substrates as electrodes for electrophoretic deposition (EPD) in which titania is

deposited from a stable colloid suspension by an electric field. The EPD technique involves two processes: electrophoresis and deposition. Electrophoresis is the motion of dispersed particles in a fluid under the influence of an electric field that is spatially uniform. The other process, deposition, is the coagulation of particles into a dense mass on substrates [12]. A procedure for growing TiO_2 films anodically was reported by Aphesteguya *et al.* [14], and their results show that at low (6 V) and high (30 V) voltages, TiO_2 is porous; the best results were obtained at voltages between 12 and 18 V.

Experimental Procedure

Titanium-coated Corning glass substrates were prepared by sputtering, in order to obtain a conductive substrate, using an INTERCOVAMEX TE12P at 100 W for 5 min. Starting material for the preparation of precursor sol of TiO_2 was Aldrich $Ti(O\text{-}n\text{-}Bu)_4$. A 0.010 M solution of $Ti(O\text{-}n\text{-}Bu)_4$ was prepared, using ethanol as solvent. The solution was then stirred at room temperature for 1 hour. A 1.4 M solution of NH_4OH in deionized water was added to the $Ti(O\text{-}n\text{-}Bu)_4$ solution and the mixture was stirred at room temperature for 2 hours, resulting in a precursor sol of TiO_2 particles at pH = 10. This sol was used in the preparation of TiO_2 films by EPD. Titanium-coated glass substrate and a stainless steel plate were used as working and counter electrodes, respectively. A constant voltage was applied between the two electrodes. After electrophoresis, the deposited substrates were withdrawn from the sol, and then they were dried at room temperature for 1 hour, and finally, consolidated for 2 hours at 400 or 500 °C. The experimental conditions for each experiment are shown in Table 1.

The colonies of *E. coli* were cultured as follows:

1. Liquid medium of Luria-Bertani (LB) for culture preparation
2. LB-Agar medium for solid culture preparation on Petri dishes
3. *E. coli* pre-inoculum preparation: sown on surface, extension and striation on LB-Agar plates and incubated for 18 hours at 37°C in a Shel-lab 1545 incubator: *E. coli* colony added to 4 ml of LB, incubated and simultaneously stirred at 200 rpm for 18 hours at 37°C, in an Orbit incubator.
4. Before the introduction of titania film into the inoculum, titania films were irradiated with 220 nm UV light with a Stratagene UV Stratalinker 2400 lamp, for 15 minutes, in order to activate the titania photocatalyst surface.
5. Inoculum preparation to probe the films' biocide activity: Two inoculums were prepared in Erlenmeyer flasks with 28 mL of LB media and 200 μL of pre-inoculum. Titania films on glass were introduced into these flasks. This was defined as time zero. Aliquots from the solutions were taken every 30 minutes. A third inoculum with no film was also prepared as a control.

Table 1. Experimental conditions

Number of experiment	Voltage [V]	Deposition time [min]	Temperature of consolidation [°C]
1	10	10	500
2	10	10	400
3	10	3	500
4	10	3	400
5	5	10	500
6	5	10	400
7	5	3	500
8	5	3	400

6. The aliquots taken from the solutions were added to a tube containing 2.5 mL of LB, and 20 µL samples were taken from these tubes to obtain a 1:5000 solution. These were seeded on LB-Agar Petri dishes. This procedure was carried out for all three inoculums.
7. All Petri dishes were incubated for 18 hours at 37°C
8. Finally, the number of colony formation units (CFU) was counted. A CFU is a bacterium that has formed a colony by reproduction. The colony is maintained as an aggregate; it is formed of thousands of bacteria, and is macroscopic and easily identified.

Results and Discussion

(1) Characterization of the films by scanning electron microscopy (SEM)

As it has been shown in Table 1, the number of experiments or samples was 8. Coating morphology and composition of each sample was analyzed by scanning electron microscopy (SEM) and EDS. SEM micrographs at low magnifications (200x) are presented in Figure 1. The images show that the best conditions for obtaining a homogeneous titania film were those of experiment 7 (Fig. 1(g)).

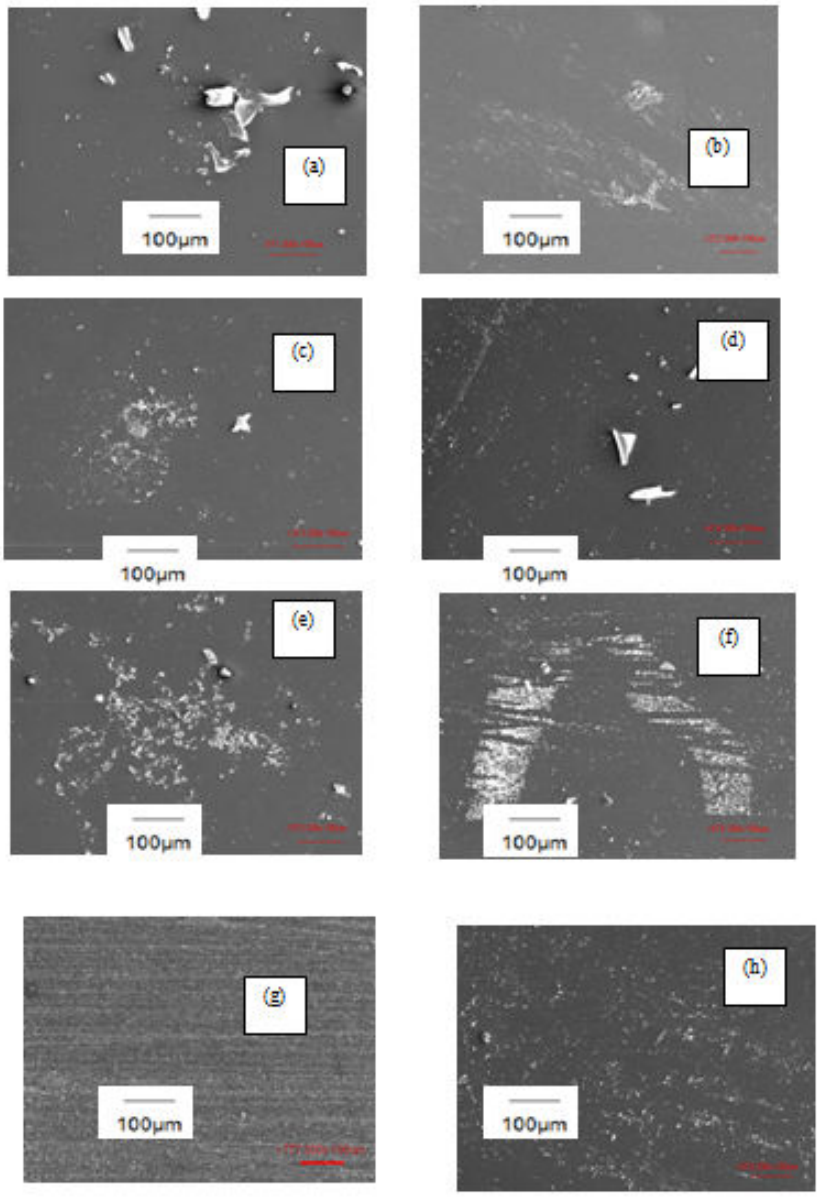

Fig. 1. SEM graphs at 200x of the samples prepared in the experiments: (a) 1, (b) 2, (c) 3, (d) 4, (e) 5, (f) 6, (g) 7 and (h) 8.

Figure 2 shows the nanostructure of sample 7 at higher magnifications, consisting of TiO_2 nanoparticles forming spherical aggregates, with some well-distributed spherical closed pores. Electrophoretic deposition conditions for this sample were 5 V for 3 min, and the consolidation temperature was 500 °C. In Fig. 2(b), some incipient can be observed in the neck formation between some particles. In the other seven cases, the conditions were not adequate for film formation, and only a few "isolated aggregates" of TiO_2 were deposited on the film matrix.

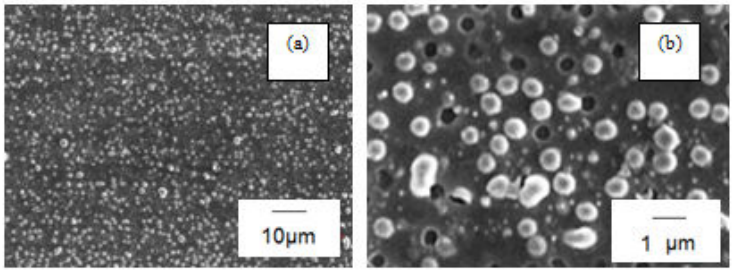

Fig. 2. SEM micrographs of sample 7: (a) at 1000x, showing a uniform film covering the glass substrate; (b) at 5000x, showing the titania spherical aggregates and well-distributed spherical closed pores forming the film.

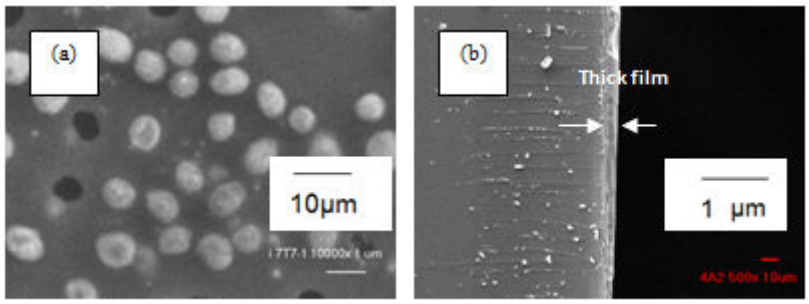

Fig. 3. SEM micrographs of sample 7: (a) at 10000x where the aggregates can easily be identified on the surface of the film. There is a coating with particles smaller than these clusters, at the back of them; (b) cross-section of the film, showing the thickness.

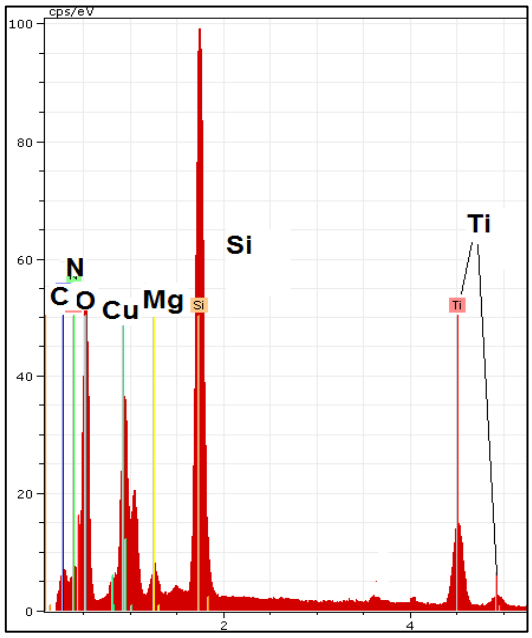

Fig. 4. EDS spectrum of the sample 7. Peaks corresponding to titanium and oxygen are present in addition to the peaks corresponding to the glass substrate.

Figure 3 (a) shows spherical aggregates on the surface of the film and their morphology. Pores are black and the grey coating formed by nanoparticles covers the substrate. In Figure 3 (b) a micrograph of the cross-section shows the thickness of film from experiment 7, which was estimated to be around 2.5 μm. This thickness corresponds to the sintered sample. Microanalysis by EDS was carried out for all samples. Peaks corresponding to oxygen and titanium were recorded only for experiment 7. EDS spectrum of sample 7 is shown in Figure 4. The other elements recorded were: Si, Ca and Mg, corresponding to glass substrate, Cu and C from sample holder, and N from air.

(2) Characterization of the samples by Atomic Force Microscopy (AFM)

Topography and roughness of the films were analyzed by AFM. Atomic force microscope used (Q-Scope, Quesant) allowed the acquisition of images using dynamic and touch modes, and optical visualization of surface at the same time.

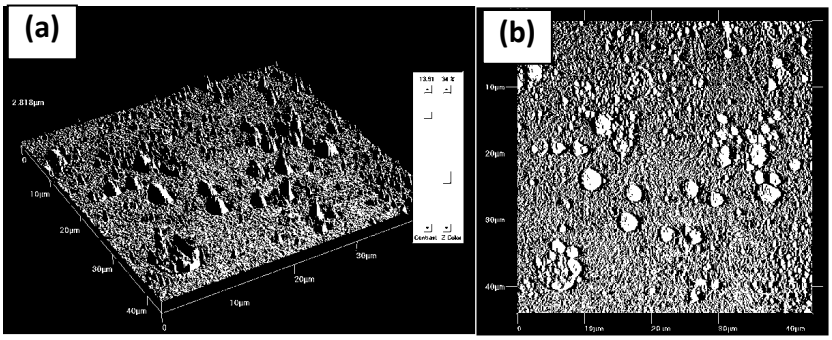

Fig. 5. AFM images of (a) the surface of sample 7T7, 3D view, and (b) top view of the surface of sample 7.

In Fig. 5, the AFM images show that some isolated aggregates (that were certainly formed by nanometric TiO_2 particles) were deposited on the nanometric homogeneous TiO_2 film matrix. In Fig. 6, profilometry of the film and surface roughness is shown, with a mean value of about 677 nm. This value is high because of aggregates present on the surface in sampling areas of 40 x 40 μm (1600 μm^2).

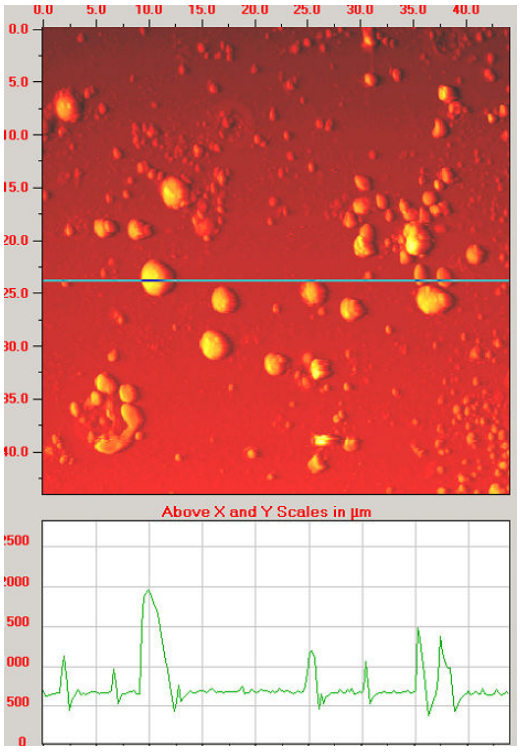

Fig. 6. AFM image and profilometry, showing the surface height of the aggregates and the film.

(3) Grazing incidence X-ray diffraction

The crystalline structure of sample 7 was determined by grazing incidence X-Ray diffraction (XRD), using Co radiation. XRD pattern of sample 7 is shown in Fig. 7. The main anatase phase reflections were recorded; peaks are broad, indicating the incipient crystallization and low crystalline size of anatase phase reached at 500 °C of thermal consolidation treatment.

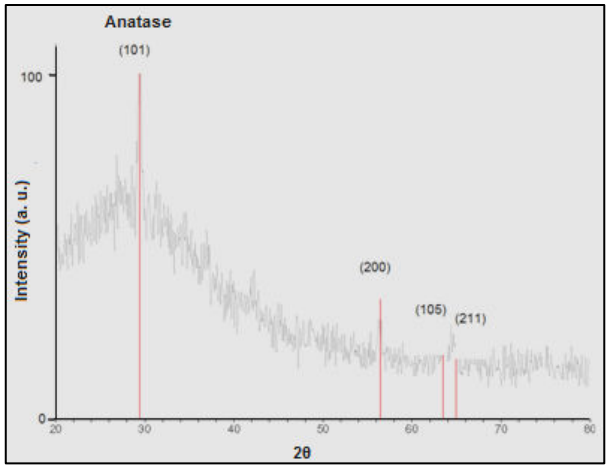

Fig. 7. XRD pattern of the anatase film, for sample 7. The main anatase reflection planes are indicated.

(4) Photocatalytic and biocide activity

Table 2 shows the biocide activity of TiO_2 films, where C+ corresponds to bacterial culture without the titania-coated glass, i.e. the control system. E.1 and E.2 correspond to two bacterial cultures grown in the same conditions as those for C+, in which the biocide activity of TiO_2 films was tested. These values correspond to the number of CFUs for each sampling point. The results show that TiO_2 films effectively reduced the number of colonies in E.1 and E.2 bacterial cultures compared to the control system. Biocide activity of the films for different times was carried out under same experimental conditions as given in Table 2.

Table 2. Results of the biocide activity.

Time[min]	C+	E.1	E.2
0	26	4	5
30	29	18	18
60	34	11	16
90	36	11	14
120	46	11	13
150	50	6	9

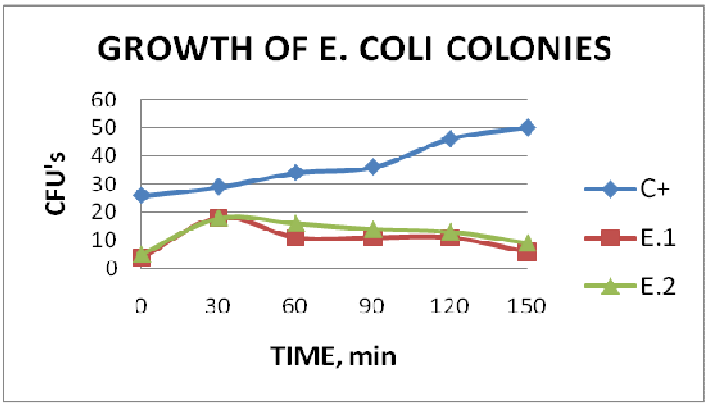

Fig. 8. Number of CFUs Vs time, showing the growth of *E. coli* colonies.

Figure 8 shows that the number of CFUs in C+ increased as a function of time due to cell division of *E. coli* bacteria. On the other hand, the two experiments in which titania-coated glass was introduced into Erlenmeyer flask, i.e. E.1 and E.2, showed a decrease in the number of CFUs. Even at time zero there were differences between the number of CFUs in the "reference system (C+)" with respect to experiments E.1 and E.2, which indicates that titania inhibits cell division immediately. Interestingly it seems that titania has two different effects: for the first 60 minutes it is bacteriostatic, which means that it inhibits the division of *E. coli* bacteria; and for the last 90 minutes, it is a bactericide, which means that the cell walls are destroyed because of high oxidative power of titania catalysts. It is important to emphasize that for inoculums E.1 and E.2, the titania-coated glass was irradiated with UV light before it was introduced into the flasks. If the inoculums were irradiated after the introduction of films into flasks, the wall of bacteria would be destroyed by irradiation and not by the effect of titania catalyst, since the energy corresponding to UV wavelength is very high. Therefore, the reduction in number of CFUs is attributed only to titania catalyst effect.

(5) Transparency of the films

Films with high transparency have a wide variety of applications, in the form of self-cleaning lenses and windows, and for solar energy conversion, treatment of pollutants, and of course degradation of microorganisms. The TiO_2 films prepared by combined methods were completely transparent. To prove this, Fig. 9 and Fig. 10 show photographs of optically transparent titania film on a Corning glass substrate on a transparent centimeter ruler.

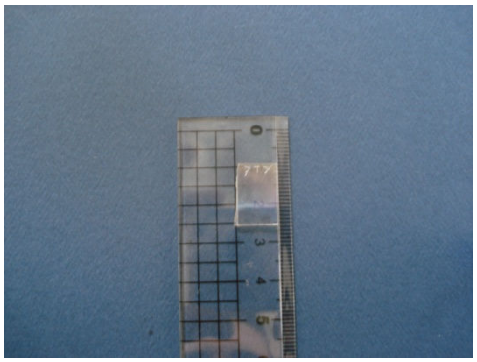

Fig. 9. Optical transparency of the titania films on a ruler.

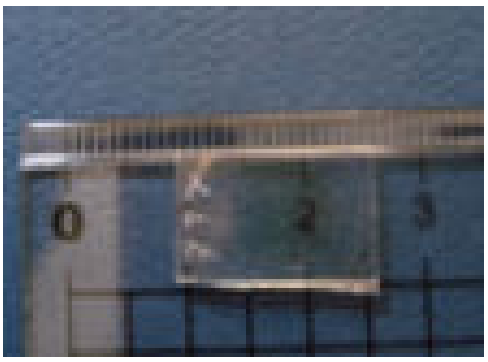

Fig. 10. Picture showing the dimensions of sample 7 (1.5 x 1.0 cm).

Conclusions

1. The combination of sputtering and electrophoretic deposition techniques enabled us to obtain transparent and nanostructured films of anatase.

2. TiO_2 film was only obtained in the deposition conditions of experiment 7.

3. The obtained anatase films had high biocide activity. Number of CFUs was dramatically decreased when titania-coated glass was introduced into bacterial cultures. The number of CFUs in experiments E.1 and E.2, which contained titania films, was 6 and 9, at 150 minutes, respectively; number of CFUs in the inoculum without titania film was 50. TiO_2 anatase phase films have an inhibitory effect on *E. coli*, and from the results, it was concluded that they have two different effects: bacteriostatic, they inhibit the division of *E. coli* bacteria; and bactericidal, which means that wall of the cells is destroyed because of high oxidative power of titania catalysts.

4. The obtained titania films were optically transparent, which makes them adequate for several technological applications, such as self-cleaning lenses and windows, solar energy conversion, treatment of pollutants, and of course microorganism degradation.

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References

[1] N.S. Allen, M. Edge, G. Sandoval, J. Verran, J. Stratton and J. Maltby: Photochemistry and Photobiology 81 (2005)279-290.

[2] J. Xu, X. Zhao, J. Du and W. Chen: Science and Technol. Vol. 17 (2000), p. 163-176.

[3] F. Gordillo-Delgado, J.G. Mendoza-Álvarez and O. Zelaya-Ángel: Revista Colombiana de Física 38 1 (2006)129-132.

[4] M.-J. López-Muñoz, R. van Grieken, J. Aguado and J. Marugan: Catalysis Today 101 (2005) 307-314.

[5] A. Mills, G. Hill, M. Crow and S. Hodgen: Journal of Applied Electrochemistry 35 (2005) 641-653.

[6] K.T. Meilert, D. Laub and J. Kiwi: J. of Molecular Catalysis A: Chemical Vol. 237 (2005) 101-108.

[7] J. Sun, L. Gao and Q. Zhang: Journal of the American Ceramic Society 86 (2003) 1677-1682.

[8] M.A. Rivero, N.L. Padola, A.L. Etcheverria and A.E. Parma: Medicina (Buenos Aires) 64 (2004) 352-356.

[9] D.S. Kim and S.-Y. Kwak: Environ. Sci. Technol. 43 (2009) 148-151.

[10] J. Rathousky, M. Slabova, K. Macounová and A. Zukal: Microp. and Mesop. Mater. 51 (2002) 599-603.

[11] K. Takahashi: Journal of the American Ceramic Society 89 10 (2006)3107-3111.

[12] P. Sarkar and P.S. Nicholson: Journal of the American Ceramic Society 79 (1996) 1987-2002.

[13] J.M. Albella, I. Jiménez, C. Gómez-Aleixandre and A. Alberdi: Boletín de la Sociedad Española de Cerámica y Vidrio 46 4 (2007)171-176.

[14] J. Aphesteguya and G.S. Duffo: IV Coloquio Latinoamericano de Fractura y Fatiga (2000), pp. 759-765.