

# RSC Advances



This is an *Accepted Manuscript*, which has been through the Royal Society of Chemistry peer review process and has been accepted for publication.

*Accepted Manuscripts* are published online shortly after acceptance, before technical editing, formatting and proof reading. Using this free service, authors can make their results available to the community, in citable form, before we publish the edited article. This *Accepted Manuscript* will be replaced by the edited, formatted and paginated article as soon as this is available.

You can find more information about *Accepted Manuscripts* in the [Information for Authors](#).

Please note that technical editing may introduce minor changes to the text and/or graphics, which may alter content. The journal's standard [Terms & Conditions](#) and the [Ethical guidelines](#) still apply. In no event shall the Royal Society of Chemistry be held responsible for any errors or omissions in this *Accepted Manuscript* or any consequences arising from the use of any information it contains.

# The Role of Silver Nanoparticles Functionalized on TiO<sub>2</sub> for Photocatalytic Disinfection of Harmful Algae

Cite this: DOI: 10.1039/x0xx00000x

Soo-Wohn Lee,<sup>a</sup> S. Obregón,<sup>b,c</sup> and V. Rodríguez-González<sup>d</sup>

Received 00th January 2012,  
Accepted 00th January 2012

DOI: 10.1039/x0xx00000x

www.rsc.org/

Silver loaded TiO<sub>2</sub> samples were prepared by photodeposition of different amounts of Ag<sup>+</sup> ions over commercial titanium dioxide (Evonik TiO<sub>2</sub> P25) in aqueous media without the presence of sacrificial agents. The obtained photocatalysts were characterized by several techniques such as X-ray powder diffraction (XRD), UV-Vis diffuse reflectance spectroscopy (DRS), transmission electron microscopy (TEM) and X-ray photoelectron spectroscopy (XPS) in order to correlate the effect of the silver amount on the photocatalytic properties of the final nanocomposite. The effect of the silver nanoparticles on the photocatalytic behaviour of TiO<sub>2</sub> was evaluated by means of the photodegradation of methyl orange dye and the inactivation of noxious algae *Tetraselmis suecica* and *Amphidium carterae* under continuous exposure of low power irradiation UV-light. The sample with 1.5% wt. of silver nanoparticles showed the highest photocatalytic elimination of the azo dye and both algae types. According to the results, the cells were deformed during the photocatalytic process by the attack of highly reactive species such as hydroxyl radicals, H<sub>2</sub>O<sub>2</sub> and superoxide ions generated on the TiO<sub>2</sub> surface. The algae cells were not regenerate by themselves after photocatalytic process due the high degree of fragmentation that suffered during the light irradiation.

## 1. Introduction

The algal population level of a water body is an indicator of the water pollution status.<sup>1</sup> For example, the excessive growth of the microalgae *Amphidium carterae* indicates oil pollution of water bodies.<sup>2</sup> The increase in the amount of organic matter that is added to water bodies results in an increase in the nutrient supply for the growth of microorganisms, a phenomenon known as eutrophication.<sup>2</sup> When extremely high levels of nutrients are present in the water, the microorganism blooms can lead to cell clumps which grow, die and accumulate leaving floating mats of decomposing organic matter. Sometimes, such cells release toxins which may be highly harmful to aquatic life, causing death, which in return causes serious problems to human health.<sup>3-5</sup> An emerging option for removing harmful algae bloom is the photocatalysis. The sterilization of microbial cells in water by this green photochemical process was first reported by Matsunaga and co-workers.<sup>6</sup> They used a TiO<sub>2</sub>/Pt powder catalyst which upon illumination with near-UV light kills the *Lactobacillus acidophilus*, *Saccharomyces cerevisiae*, and *Escherichia coli* bacteria. Since this report, many other studies have been reported for the successful removal of pathogenic microorganisms with TiO<sub>2</sub> powders as well as TiO<sub>2</sub> thin films.<sup>7,8</sup> For example, Linkous and co-workers studied the photocatalytic effect of TiO<sub>2</sub> and WO<sub>3</sub> coatings in the presence and absence of Pt and Ir as co-catalysts for the elimination of the filamentous *Oedogonium* algae.<sup>9</sup> These authors found that

by using a combination of black light and fluorescent lamps, a cement surface coated with TiO<sub>2</sub> was highly effective in the inhibition of the growth of *Oedogonium* algae in comparison with the uncoated cement.

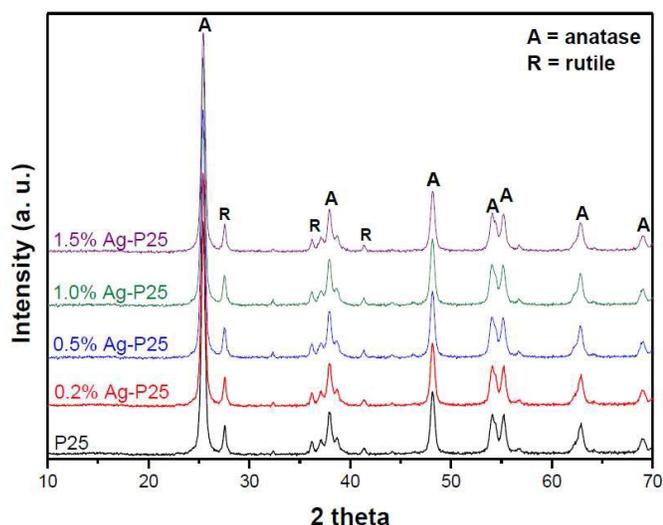
Recently, we have demonstrated the effective use of several photocatalysts for the complete removal of noxious microalgae such as *Amphidium carterae*, also known as “red tide” due to the characteristic colour of their floating dead cells.<sup>10-12</sup> Following this research line, the present work reports the photocatalytic effect on the elimination of the harmful microalgae *A. carterae* and *T. suecica* by using different amounts of Ag nanoparticles photodeposited on commercial titanium dioxide (Evonik TiO<sub>2</sub> P25) under near UV-irradiation.

## 2. Experimental

### 2.1 Preparation of silver-TiO<sub>2</sub> powder samples

Silver-TiO<sub>2</sub> loaded samples were prepared by the photo-impregnation method, which has been described previously.<sup>10</sup> For this purpose, Evonik TiO<sub>2</sub> P25 was used as the titanium dioxide source. To prepare 1 wt. % of Ag nanoparticles loaded on the TiO<sub>2</sub> surface, 0.472 g of silver nitrate (Samchun, 99.8%) were added to 1L of distilled water and placed in a ultrasound bath for 5 min in order to ensure complete disaggregation. Afterwards, 29.7 g of Evonik TiO<sub>2</sub> P25 were added to the silver aqueous solution and the slurry was stirred vigorously for 1h in order to achieve the adsorption-desorption equilibrium of Ag<sup>+</sup> ions on the TiO<sub>2</sub> surface. Then, the mixture was exposed to UV





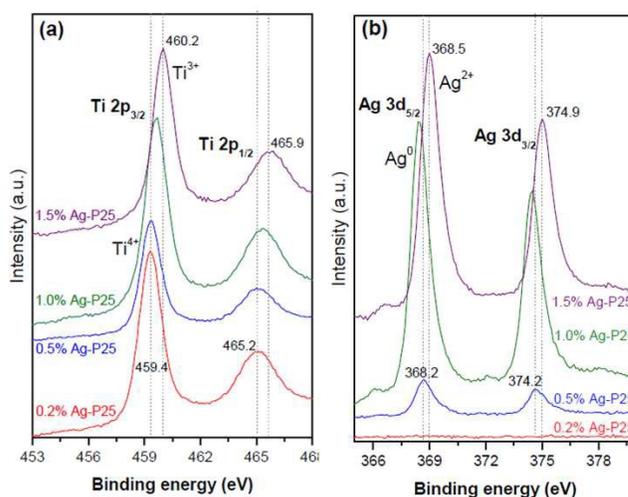
**Figure 1.** XRD patterns of the Ag loaded TiO<sub>2</sub> powders.

found due to the low concentration and/or high dispersion of the reduced metal photodeposited on the surface of the photocatalyst.

The energy band gap value for the sample without silver nanoparticles (P25) was estimated as 3.2 eV, which is the same as the one that has been reported previously.<sup>15</sup> A slight increase of the silver amount (0.2%) leads to decrease in the energy band gap and until a value of 2.98 eV is reached for the sample with 1.5% wt. of silver nanoparticles. Since photodeposition of silver nanoparticles on titanium dioxide do not change the positions of the valence and conduction band of TiO<sub>2</sub>, the differences in the band gap of the samples are could be attributed to the surface plasmon absorption of spatially confined electrons in Ag nanoparticles.<sup>16</sup> The surface area values of the samples showed no changes from the value obtained for the Evonik TiO<sub>2</sub> P25 (~54 m<sup>2</sup>·g<sup>-1</sup>). These data confirm that the presence of silver nanoparticles do not affect the textural properties of the titanium oxide.

The XPS spectra of Evonik TiO<sub>2</sub> P25 loaded with different amounts of silver nanoparticles were analyzed. The Ti 2p spectra, shows Ti 2p XPS peaks at binding energies of 459.4 and 465.2 eV, which are characteristic of the Ti<sup>4+</sup> oxidation state in TiO<sub>2</sub>, see Figure 2a.<sup>16</sup> On the other hand, Figure 2b shows the Ag 3d peaks where the binding energies for Ag agree fairly well with those reported for silver impregnated TiO<sub>2</sub> samples.<sup>16</sup> The Ag 3d peaks appear at a binding energy of 368.2 eV (3d<sub>5/2</sub>) and the 3d doublet (3d<sub>3/2</sub>) is located at approximately 374.2 eV. These binding energies indicate that a large amount of reduced silver particles exist on the TiO<sub>2</sub> support surface.<sup>17</sup> The peaks of the silver XPS spectra shows greater intensity with higher silver content. The 3d Ag peaks at 368.2 and 374.2 eV are characteristic of metallic Ag, while the shift of 3d Ag peaks at 374.9 and 368.9 eV could suggest the presence of silver particles with different sizes, which are normally assigned to the Ag<sub>2</sub>O state.<sup>17</sup>

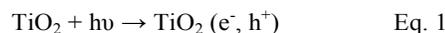
Figure 3(a-b) shows the TEM images of the samples with 1.0 and 1.5% wt. of silver nanoparticles. Samples with lower amounts of Ag nanoparticles were also examined. However, the silver nanoparticles were not observed probably due to their small sizes and low concentration. The average diameters of the anatase and rutile particles were estimated to be 25 and 85 nm,



**Figure 2.** XPS spectra of (a) Ti 2p and (b) Ag 3d core levels for the Ag-TiO<sub>2</sub> photodeposited samples.

respectively, which is consistent with the results reported by Ohno and co-workers.<sup>18</sup> The toxicity of the silver nanoparticles depends on their size, where smaller sizes ( $\leq 10$  nm) exhibit higher anti-microbial activity due to easier cell penetration.<sup>19</sup>

Particle size distribution of Ag nanoparticles was done from TEM micrographs by counting at least 300 particles from each sample, Figure 4(c-d). The results show silver nanoparticles with a very narrow size distribution and a predominant size of 4-5 nm for the sample with 1.0% wt. On the other hand, when the silver amount is 1.5%, a heterogeneous distribution is found in the 2-13 nm range with a typical size of 7-8 nm. Sclafani and Hermann proposed a mechanism that describes the deposition and growth of silver particles on the TiO<sub>2</sub> surface.<sup>20</sup> When titanium dioxide is irradiated with UV light, the formation of electron-hole pairs occurs by transferring electrons to the conduction band from the valence band as shown by Eq. 1:



Then, silver ions are initially adsorbed on the surface of TiO<sub>2</sub> particles and the photogenerated electrons reduce the Ag<sup>+</sup> ions to silver metal atoms:

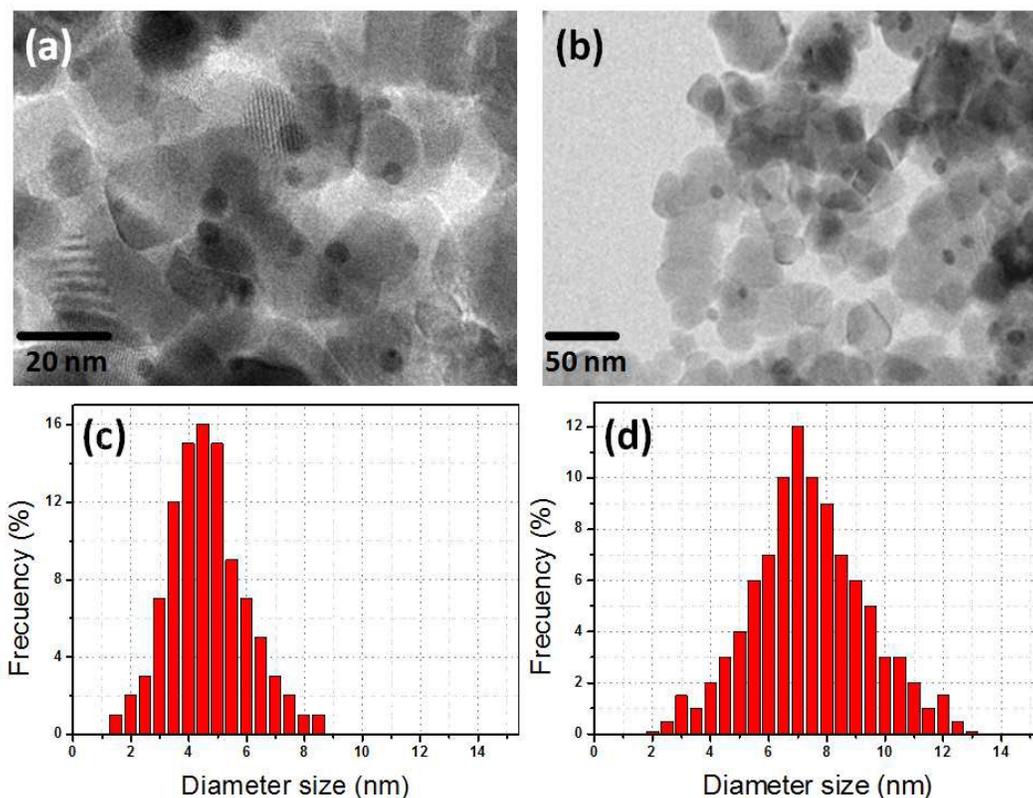


It is possible that an increase in the silver amount causes the formation of larger particles during the growth stage:



### 3.2 Photocatalytic tests

The photocatalytic activity of the Ag-TiO<sub>2</sub> samples was tested by the degradation of methyl orange dye under UV irradiation.<sup>21</sup> The temporal evolution of the dye concentration during the photodegradation process is shown in Figure 4. As can be seen, the difference in the photodegradation of methyl orange dye between bare TiO<sub>2</sub> and 0.5% wt. Ag-TiO<sub>2</sub> is small, after 210 min of UV-light irradiation. Moreover, the photodegradation was increased using the 1% wt. Ag-TiO<sub>2</sub> sample. Also, the sample 1.5% wt. Ag-TiO<sub>2</sub> showed a similar degradation of the dye. The kinetic data can be adjusted



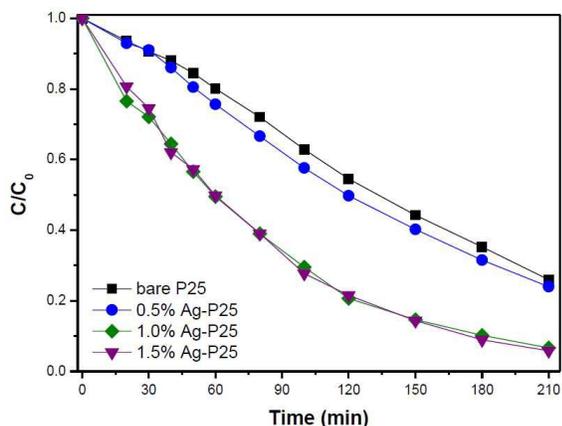
**Figure 3.** (a-b) Transmission Electron Microscopy and (c-d) particle size distribution of Evonik TiO<sub>2</sub> P25 samples impregnated with 1.0 and 1.5% of silver nanoparticles.

according with a first-order reaction equation following the Langmuir-Hinshelwood model. On the basis of this model, the half-life time for bleaching the methyl orange dye was 57.8 min for the 1.0% wt. Ag-TiO<sub>2</sub> sample compared and 132 min for the bare TiO<sub>2</sub>.

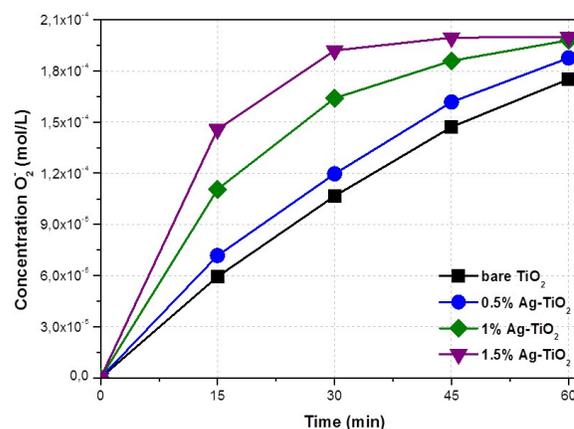
The photocatalytic production of the superoxide ion (O<sub>2</sub><sup>-</sup>) was estimated by means the specific reduction reaction of the nitroblue tetrazolium molecule (NBT) by the superoxide ions. As shown in Figure 5, the bare TiO<sub>2</sub> led to the formation of a lower concentration of superoxide ions compared with the silver loaded samples. The trapping of the electron by oxygen

molecule resulting in the formation of O<sub>2</sub><sup>-</sup> is an effective pathway for promoting the separation of the photogenerated charge carriers. Therefore, these results show that the addition of silver nanoparticles on TiO<sub>2</sub> increase its photoactivity.

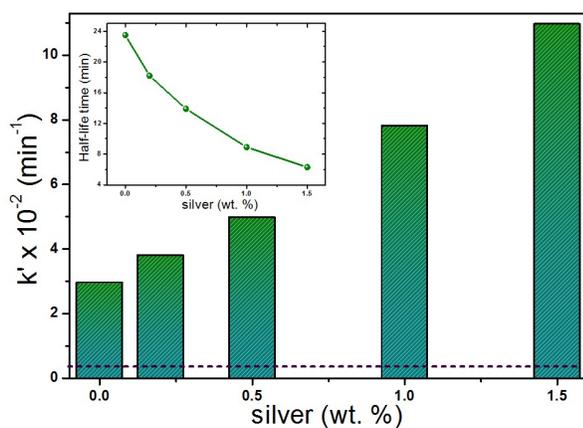
The photocatalytic performance of the photocatalysts also was evaluated for the elimination of noxious microalgae cells under UV-A irradiation. Figure 6 shows the rate constants for the photoelimination of *Tetraselmis suecica* cells in terms of the amount of Ag nanoparticles on the TiO<sub>2</sub> surface. According to the results, the increase in the silver amount promotes a considerable increase in the photocatalytic removal of the algae



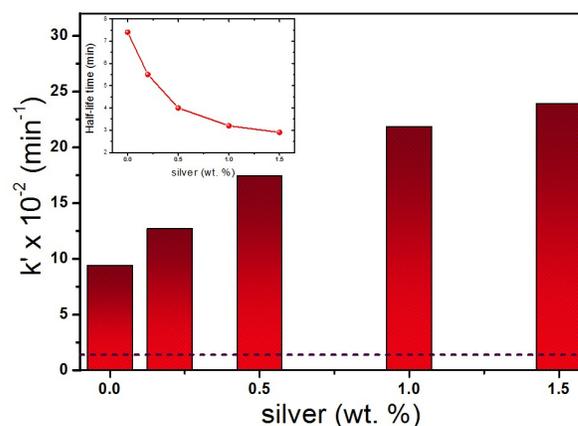
**Figure 4.** Photodegradation of methyl orange dye for the Ag loaded TiO<sub>2</sub> powders under UV-light irradiation.



**Figure 5.** Photocatalytic production of superoxide ion (O<sub>2</sub><sup>-</sup>) by Ag-TiO<sub>2</sub> samples under UV-light irradiation.



**Figure 6.** Rate constants and half-life time for *T. suecica* elimination as function of silver contents on TiO<sub>2</sub> samples.



**Figure 7.** Rate constants and half-life time for *A. carterae* elimination as function of silver contents on TiO<sub>2</sub> samples.

cells. The dashed line with a value of  $0.4 \times 10^{-2} \text{ min}^{-1}$  can be considered negligible and represents the rate constant of the system under UV light in absence of photocatalyst. In other words, the presence of UV light over *T. suecica* cells does not appear to cause damage at cellular level that could lead to the death of the algae. The top inset of Figure 6 shows the half-life time of the microalgae cells during the photocatalytic process. A semi-linear relationship is observed, which is consistent to the amount of silver nanoparticles on TiO<sub>2</sub>. A half-life time of 23.5 min was obtained for the bare TiO<sub>2</sub> sample in the absence of silver nanoparticles. In the presence of 0.2% wt. of Ag nanoparticles, the half-life time was 18.2 min and continued decreasing as the Ag concentration was increased until a half-life time of 6.3 min was obtained. This half-life time corresponds to a value almost four times higher than that obtained with bare TiO<sub>2</sub>. Normally, experiments in darkness are needed to confirm the photocatalytic effect by comparing the light versus darkness reactivity. In this particular case, the algae cells are photosynthetic and require light that their chloroplasts can absorb, increasing the concentration of living cells. However, it is clear that the presence of Ag impregnated TiO<sub>2</sub> photocatalysts under UV-light leads to a considerable decrease in the living algae cells. A comparison of micrographs showing the microalgae cells before and after the photocatalytic process indicates that photocatalysis damages clearly the structure of the cells (data not shown). The cells can be attacked by highly reactive species such as hydroxyl radicals, H<sub>2</sub>O<sub>2</sub> and superoxide ions generated on the TiO<sub>2</sub> surface. After 8h of UV irradiation, the algae cells were deformed and annihilated thus preventing regeneration. After, nutritive water (Natural Live Plankton Co., Ltd.) was added to the system and incubated at 25 °C for 72 h. The suspension was analyzed to count the cell concentration, where living cells were not observed.

On the other hand, Figure 7 shows the rate constant for the photoelimination of the microalgae *Amphidium carterae*, where the dashed line represents the rate constant in absence of photocatalyst, which can be considered negligible. It is noteworthy to mention that the silver loaded samples showed a higher photocatalytic removal of living cells when the Ag nanoparticles are at higher concentration. However, the elimination of the cells did not represent a linear relationship with respect to the silver concentration in the samples. As it can be seen in the top inset of Figure 7, the half-life times are lower than those obtained in the elimination of the microalgae *T. suecica*. It is possible that *A. carterae* cells are less resistant to

the hydroxyl radicals produced during the photocatalytic process and therefore these oxidative radicals cause damage more easily to the cell membranes, causing their death. According to Ohno and co-workers, the rutile phase in TiO<sub>2</sub> P25 powder does not exist as an overlayer on the surface of anatase particles.<sup>16</sup> When the anatase and rutile particles are put in contact with aqueous media, the mixed phases must be the key to the high photocatalytic activity of the TiO<sub>2</sub> P25 powder. On the other hand, it is well known that the antimicrobial activity of the Ag nanoparticles is dependent on its size due to the ease with which they can be attached to the surface of the cells. This adhesion disturbs the cell membrane permeability and respiration functions of cells causing deformation and subsequent death.<sup>17</sup> Nevertheless, in our case this antimicrobial activity can be considered as a minimal effect, mainly because the Ag nanoparticles are not detached from the TiO<sub>2</sub> surface. This statement can be supported by a previous study by ICP analysis, where the lixiviation of silver nanoparticles from TiO<sub>2</sub> can be considered negligible under UV irradiation conditions.<sup>8</sup> Electron transfer from the TiO<sub>2</sub> conduction band to Ag nanoparticles is thermodynamically possible because the Fermi level of TiO<sub>2</sub> is higher than that of metallic silver. This phenomenon is expected to provide the formation of a Schottky barrier at the metal-semiconductor contact region by acting as an electron trap to facilitate the charge separation of the hole-electron pair. It also promotes the interfacial electron transfer process, thereby increasing the photocatalytic activity of TiO<sub>2</sub>.<sup>19</sup> However, a high silver content may be detrimental due the TiO<sub>2</sub> surface may become be covered by the Ag nanoparticles and the absorption of photons be decreased, thereby decreasing its photocatalytic activity.

#### 4. Conclusions

Several TiO<sub>2</sub> photocatalysts with different amounts of Ag nanoparticles were prepared in aqueous medium without the presence of sacrificial agents. The effect of the photodeposition of the Ag nanoparticles on TiO<sub>2</sub> was evaluated by several model reactions such as the photodegradation of methyl orange dye, the photoproduction of the superoxide ion and disinfection tests under UV-A irradiation conditions. According to microscopic observations, the cells were divided into fragments and their intracellular components were released during the

photocatalytic process. The silver nanoparticles acts as no recombination centers since the negatively charged silver sites can attract positively charged holes, providing a high efficiency of charge separation of the hole-electron pair.

### Acknowledgements

This research has been supported by the project “*Development of Multifunctional Nanomaterials and Processing Technology for Eco-friendly Applications*” from The National Research Foundation of Korea. We also thank the National Institute for Nanotechnology, located at CIMAV-Chihuahua, Mexico for the TEM characterization.

### Notes and references

<sup>a</sup> Department of Environmental Engineering, Sun Moon University, Galsan-Ri, Tangjung-Myon, Asan Chungnam 336-708, South Korea.

<sup>b</sup> Facultad de Ciencias Físico Matemáticas, Universidad Autónoma de Nuevo León, Av. Universidad S/N, San Nicolás de los Garza, 66450 Nuevo León, México.

<sup>c</sup> Instituto de Ciencia de Materiales de Sevilla, Centro Mixto Universidad de Sevilla-CSIC. C/Américo Vespucio, 49, 41092 Sevilla, Spain. E-mail: [sergio.obregon@icmse.csic.es](mailto:sergio.obregon@icmse.csic.es) Tel: +34 954489536 ext. 909225

<sup>d</sup> División de Materiales Avanzados, IPICYT (Instituto Potosino de Investigación Científica y Tecnológica), Camino a la Presa San José 2055 Col. Lomas 4a. sección C.P. 78216, San Luis Potosí, S.L.P., México E-mail: [vicente.rdz@ipicyt.edu.mx](mailto:vicente.rdz@ipicyt.edu.mx) Tel: +52 44483 42000 ext. 7295

1 B.A. Markert, A.M. Breure, H.G. Zechmeister, Trace Metals and other Contaminants in the Environment 6: Bioindicators and biomonitors, Elsevier, 2003.

2. S. K. Agarwal, Environmental Monitoring, APH Publishing, India, 2005.

3. G. Honsell, M.D. Bortoli, S. Boscolo, C. Dell’Aversano, C. Battocchi, G. Fontanive, A. Penna, F. Berti, S. Sosa, T. Yasumoto, P. Ciminiello, M. Poli, A. Tubaro, *Environ. Sci. Technol.* 2011, **45**, 7051.
4. N. McQuaid, A. Zamyadi, M. Prévost, D.F. Bird, S. Dorner, *J. Environ. Monit.* 2011, **13**, 455.
5. R. Dai, H. Liu, J. Qu, J. Ru, Y. Hou, *J. Hazard. Mat.* 2008, **153**, 470.
6. T. Matsunaga, R. Tomoda, T. Nakajima, H. Wake, *FEMS Microbiol. Lett.* 1985, **29**, 211.
7. K. Gupta, R.P. Singh, A. Pandey, A. Pandey, *Beilstein J. Nanotechnol.* 2013, **4**, 345.
8. S. Khan, I.A. Qazi, I. Hashmi, M.A. Awan, N.S. Zaidi, *J. Nanomater.* 2013, 2013, Article ID 531010.
9. C.A. Linkous, G.J. Carter, D.B. Locuson, A.J. Ouellette, D.K. Slattery, L.A. Smitha, *Environ. Sci. Technol.* 2000, **34**, 4754.
10. V. Rodríguez-González, S.O. Alfaro, L.M. Torres-Martínez, S.H. Cho, S.W. Lee, *Appl. Catal. B: Environ.* 2010, **98**, 229.
11. S.O. Alfaro, A. Martínez-de la Cruz, L.M. Torres-Martínez, S.W. Lee, *Catal. Commun.* 2010, **11**, 326.
12. S.W. Lee, S. Obregón-Alfaro, V. Rodríguez-González, *J. Photoch. Photobio. A* 2011, **221**, 71.
13. V. Rodríguez-González, S. Obregón-Alfaro, L.M. Lozano-Sánchez, Soo-Wohn Lee, *J. Mol. Catal. A: Chem.* 2012, **353-354**, 163.
14. X. Xu, X. Duan, Z. Yi, Z. Zhou, X. Fan, Y. Wang, *Catal. Commun.* 2010, **12**, 169.
15. S. Obregón Alfaro, V. Rodríguez-González, A.A. Zaldivar-Cadena, S.W. Lee, *Catal. Today* 2011, **166**, 166.
16. S. Saha, J.M. Wang, A. Pal, *Sep. Purif. Technol.* 2012, **89**, 147.
17. X.G. Hou, M.D. Huang, X.L. Wu, A.D. Liu, *Chem. Eng. J.* 2009, **146**, 42.
18. T. Ohno, K. Sarukawa, K. Tokieda, M. Matsumura, *J. Catal.* 2001, **203**, 82.
19. L. Kvítek, A. Panáček, J. Soukupová, M. Kolář, R. Večeřová, R. Prucek, M. Holecová, Zbořil. Radek, *J. Phys. Chem. C* 2008, **112**, 5825.
20. A. Sclafani, J.M. Herrmann, *J. Photoch. Photobio. A* 1998, **113**, 181.
21. Ch. Girginov, P. Stefchev, P. Vitanov, Hr. Dikov, *J. Eng. Sci. Tech. Rev.* 2011, **5**, 14.
22. A. Henglein, *J. Phys. Chem.* 1979, **83**, 2209.