Proceedings of the World Congress on New Technologies (NewTech 2015)
Barcelona, Spain – July 15 - 17, 2015
Paper No. 414

Antibacterial Activity of Ag/TiO2 Nanocomposite Films on Ceramic Plate

Yaw-Nan Shieh, Jung-Jie Huang
Department of Materials Science and Energy Engineering, Ming Dao University,
369, Wen-Hua Rd., Peetow, Changhua 5345, Taiwan
ynshieh@mdu.edu.tw; jjhuang@mail.dyu.edu.tw

Jian-Hua Chen
Fu Jian University of Technology

Abstract- This study investigated the antibacterial activity of ceramic plate coated with Ag/TiO_2 Nanocomposite films. Ag and TiO_2 were synthesized through; iquid chemical reduction and microwave-assisted synthesis. Ag/TiO_2 nanocomposite films were dip-coated on ceramic plate. Ag and TiO_2 nanoparticles were characterized using x-ray diffraction, scanning electron microscopy, and ultraviolet–visible absorption spectroscopy. The obtained Ag/TiO_2 composite films were applied as antibacterial films, and the antibacterial and bacteriostatic effects of the Ag/TiO_2 composite films to atmospheric bacteria were qualitatively and quantitatively experimentally investigated. The results show that the fabricated films are effective of bactericides and bacteriostats.

Keywords: nanosilver, microwave synthesis, antibacterial properties, nano-TiO_2, Ag-TiO_2 composite films

1. Introduction

Humans are frequently infected by environmental microorganisms such as bacteria, mold, yeast, and viruses. Antibacterial material research has intensively focused on various natural and inorganic substances, such as tea extracts, chitosan, silver, copper, and zinc [1–3]. Among such substances, nano-TiO_2 and Ag exhibit powerful antibacterial activity [4].

Several researchers have attempted to clarify the bacteriostatic effects of silver. Heavy metals react with proteins by combing the –SH groups of enzymes, leading to protein inactivation [5]. TiO_2 is nontoxic and has been applied in environmental treatments, such as water and air purification, water disinfection, and sterilization, because of certain unique properties such as strong photocatalytic activity and chemical stability [6]. Several studies have elucidated the photocatalytic mechanism of TiO_2 [7–9], and the most widely accepted mechanism is the migration of valence electrons to the conduction band and the formation of hole–electron pairs. These hole–electron pairs react with the molecules adsorbed on the semiconductor surface, resulting in adsorbate degradation [10]. TiO_2 kills microorganisms and is therefore used as a biocide [11–14]. However, TiO_2 has major limitations such as UV irradiation and recombination of hole–electron pairs within nanoseconds.

In recent years, several researchers have studied the combined effect of Ag and TiO_2 on antibacterial activity [15–17]. Brook et al. [18] studied the antibacterial activity of Ag-coated TiO_2 particles against *Escherichia coli* and *Staphylococcus aureus*. In addition, they found that photoreduced Ag^+ is significant in the antibacterial activity of Ag-coated TiO_2. Zhang [19] observed the formation of "pits" that damaged *E. coli* cell membranes, causing cell death. Ag nanoparticles were found in the cell membranes, and an Ag and TiO_2 composite functioned as a bioactive film. Composite films fabricated through chemical vapor deposition exhibited both self-regeneration and destruction of bacteria [20].

Several techniques, such as the sol–gel method, high-temperature glass fusion, ion implantation, ion exchange, and sputtering, have been used for obtaining Ag and TiO_2 nanoparticles [21–24]. The sol–gel method in particular has several advantages, such as high purity, ultra-homogeneity, low processing temperatures, and the possibility of fabricating new glass compositions. However, sol–gel precursor costs are higher than those of other methods.

In this study, Ag nanoparticles were prepared through microwave synthesis. Microwave synthesis has strong penetrability and uniform heating and affords quick and easy particle nucleation. Compared with traditional heating methods, microwave irradiation is simpler and faster, requires less energy, and is safer for the environment.

In this study, TiO_2 sol was prepared using the peroxide sol–gel method with some modifications. This research focused on preparing nanosized anatase TiO_2 particles by using $TiCl_4$ in neutral medium. Using this method, nanosized TiO_2 crystals were obtained at low temperatures, which is advantageous because the calcination required at high temperatures is eliminated. In addition, using H_2O_2 as the oxidizing agent affords sol in a neutral solution, rather than in the acidic conditions afforded by acid hydrolysis [25].

Ceramic plate is used to produce dishware, and microorganisms such as bacteria, yeast, and virus can easily adhere to its surface. Ag and TiO_2 nanoparticles together exhibit powerful antiseptic properties; nanosilver can kill over 650 types of bacteria in minutes. In addition, broad-spectrum sterilization, which also kills antibiotic-resistant bacteria, and nanosized TiO_2 under ultraviolet irradiation are effective antiseptics; both are nontoxic and do not irritate the skin, and bacteria do not become resistant to them.

2. Experimental

2. 1. Materials

All reagents used in the study were of analytical grade. Polyvinylpyrrolidone (PVP, molecular weight 10,000–70,000) was purchased from GAF Chemicals. $AgNO_3$ (Katayama Chemicals) was used to prepare Ag nanoparticles. $TiCl_4$ (99.6%; Alfa Aesar, Ward Hill, MA) was used as the precursor for TiO_2 solution synthesis. Atmospheric bacteria were used as model microorganisms for testing bacterial inactivation.

2. 2. Preparation Of Nanoscale Ag Particles

Ag was synthesized using liquid chemical reduction and microwave irradiation. First, $AgNO_3$ and PVP in a predetermined amount were dissolved in DI water. The obtained solution was continuously stirred at room temperature for 60 min to ensure complete dissolution of $AgNO_3$. Subsequently, the solution was irradiated in a home microwave at 260 W for 10 min.

2. 3. Preparation Of Nanoscale Tio_2 Particles

For TiO_2 sol synthesis, $TiCl_4$ was slowly added to distilled water in an ice bath (0 °C) with constant stirring for 30 min until complete dissolution. Next, aqueous NH_4OH (30 wt. %) was added to form white TiO_2 gel, that is, $Ti(OH)_4$. After aging for 8 hours, the hydrated TiO_2 gel was filtered and washed with distilled water until no chloride ions were detected in the solution. Distilled water was subsequently added to the hydrated TiO_2 white gel cake and dissolved to a 1%–2% concentration. Finally, aqueous H_2O_2 was added to oxidize the gel and form a titanium peroxide solution, which was allowed to rest for 1 hour.

The resulting aqueous titanium peroxide solution was microwave irradiated at 350 kW for 10 min. Thus, transparent anatase TiO_2 sol was obtained.

2. 4. Preparation Of Ag/Tio_2 Composite Thin Films

Ag/TiO_2 thin films were prepared by mixing Ag and TiO_2 solutions of different $Ag:TiO_2$ ratios and dip-coating them on ceramic plate. After drying, the coated samples were heated in a hot muffle furnace at 300 °C for 15 min.

2. 5. Antibacterial Activity

The antibacterial effects of the synthesized Ag and TiO_2 on atmospheric bacteria were initially investigated through controlled experiments. In the controlled experiments, bacterial cultures were first incubated in a lactobacillus nutrient solution for a day. The bacterial culture samples were then coated on the ceramic plate coated with Ag/TiO_2 films. After 2 hours, the remaining bacterial cultures were collected and incubated in the lactobacillus nutrient solution again. To evaluate the antibacterial effect, the number of bacterial cluster remaining on plates was counted

3. Results and Discussion

3. 1. X-Ray Diffraction And Scanning Electron Microscopy Analyses

Figure 1 shows the X-ray diffraction (XRD) pattern of the crystal structure of pure TiO_2 thin film formed using the microwave–hydrothermal method. XRD analyses confirmed the formation of high-purity anatase TiO_2 films synthesized using the microwave-prepared colloidal suspension and dip-coated after annealing in air at 550 °C for 2 h the main peak at $2\theta = 25.08°$ clearly shows the formation of (101) anatase TiO_2. A small peak at $2\theta = 27.93°$ is attributable to the (110) plane of rutile TiO_2.

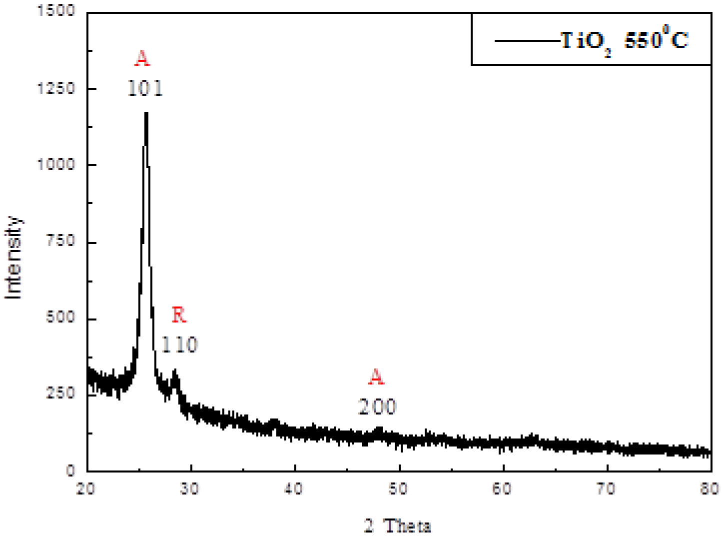

Fig. 1. XRD spectra of TiO2 thin film after microwave hydrothermal and annealed process.
A: anatase phase   R; rutile phase

Figure 2 presents a scanning electron microscopy (SEM) image of pure TiO_2 nanosized particles synthesized using microwave irradiation and annealing in air at 550 °C for 2 h. The nanocrystalline TiO_2 grains are uniform and without aggregation after annealing in air at 550 °C. The average grain size is 13.4 ± 2.5 nm, which is in the nanocrystalline range that provides the maximal life cycle of excited electrons.

3. 2. Ultraviolet–Visible Spectroscopy Analysis

The optical absorption spectrum of nanosilver particles is shown in Fig. 3. Usually, the absorption peak of nanosized particles is blue-shifted. In addition, the particle size distribution broadens as the area and the half-high width of the absorption peak increase. Moreover, a change in only the height and not the location and width of the absorption peak implies an increased nanoparticle concentration [26].

As shown in Fig. 3, the peak centers of the two curves are at approximately 415 nm, which is attributable to the plasma resonance of the silver surface. The lower curve represents the silver colloids formed before microwave irradiation. The lower absorption peak may be attributable to the PVP surfactant capsules on the sliver particle surface, which inhibit reactions, particularly at low temperatures.

However, after microwave irradiation, the absorption peak clearly increased, possibly because the irradiation promoted silver particle formation.

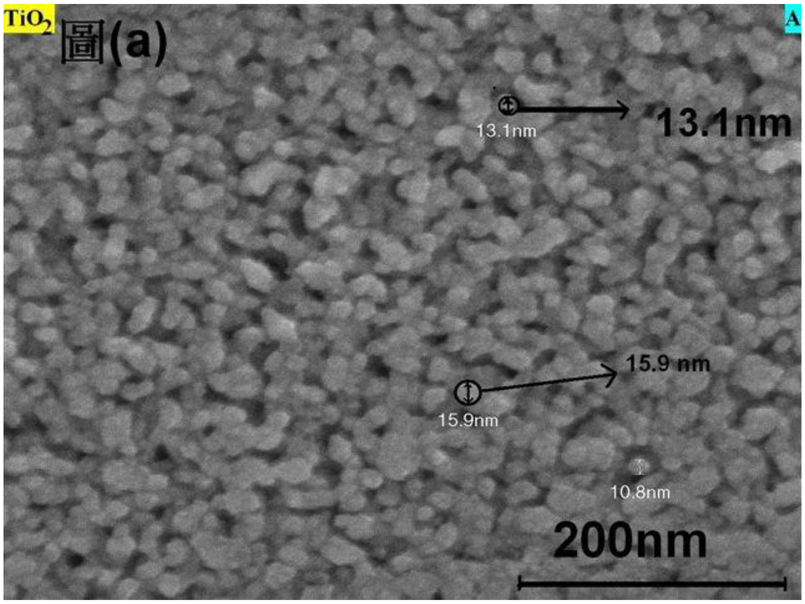

Fig. 2. Scanning electron micrographs of TiO_2 nanoparticles

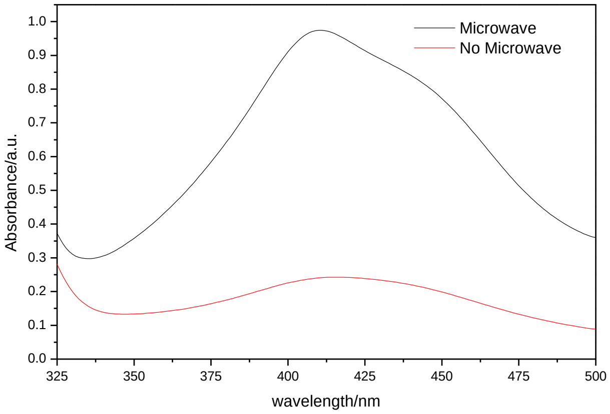

Fig. 3. Nano silver ultraviolet-visible absorption spectrum

The morphology of nanosized Ag is depicted in Fig. 4. The nanoparticles are visibly spherical, with diameters ranging from 13 to 20 nm. The particles are sufficiently separated and homogeneously distributed over the samples.

3. 3. Antimicrobial Test

The bactericidal activity of TiO_2, Ag, and Ag/TiO_2 thin films was investigated using atmospheric bacteria in the dark and under visible light irradiation, as shown in the following figures. A blank glass substrate was used as the control.

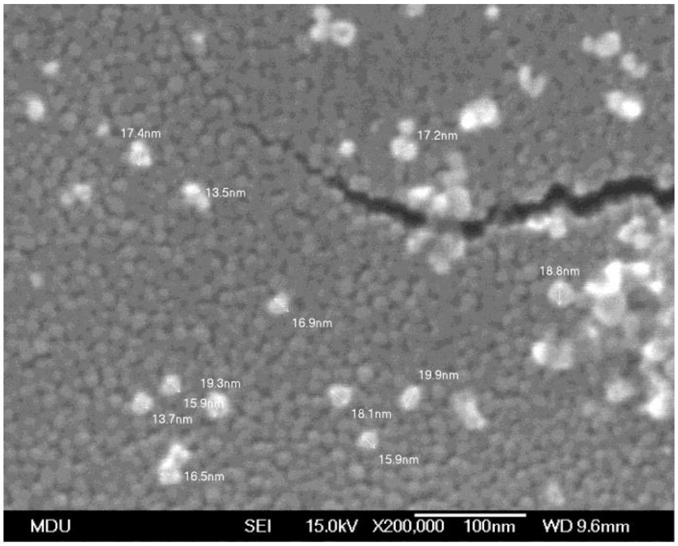

Fig. 4. Scanning electron micrographs of silver nanoparticle

Table. 1. Sample compositions.

Samples No.	1	2	3	4
Antibacterial material	Ag	TiO_2	TiO_2 /Ag	None

Figure 5 reveals the antibacterial properties of the tested substances. Clearly, samples 1 and 3, which contain Ag, exhibit high antibacterial activity.

The TiO_2 thin films exhibit weak antibacterial activity, with some bacterial colonies persisting.

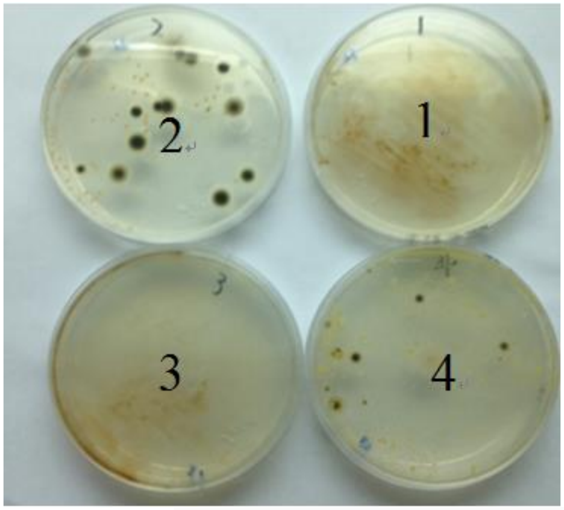

Fig. 5. The photograph of antibacterial properties of different materials.

Table. 2. Ag/TiO_2 ratios of various samples.

Samples No.	5	6	7	8
Ag/ TiO2 ratios	1:9	3:7	1:1	none

Figure 6 depicts photographs illustrating the antibacterial effect of various films in the dark for 5 min.

Clearly, an increase in Ag content remarkably increases antibacterial activity.

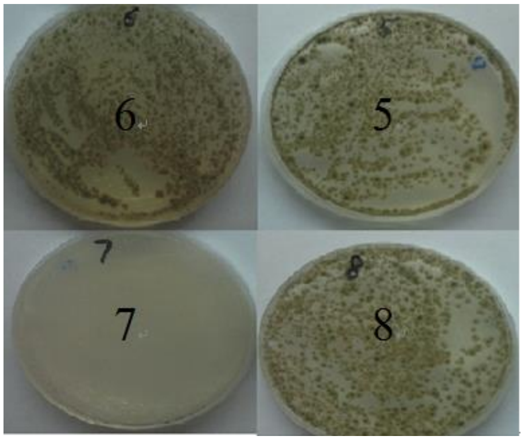

Fig. 6. The photograph of antibacterial effect of various films under the dark condition for 5min.

Table. 3. Ag/TiO_2 ratios of various samples.

Samples No.	9	10	11	12
Ag/ TiO_2 ratios	1:9	3:7	1:1	none

Figure 7 depicts photographs of bacterial growth after visible-light irradiation for 1 h in the presence of various antibacterial substances. Clearly, no bacterial colony survived the visible-light irradiation. Only on the control sample did a few bacterial colonies persist.

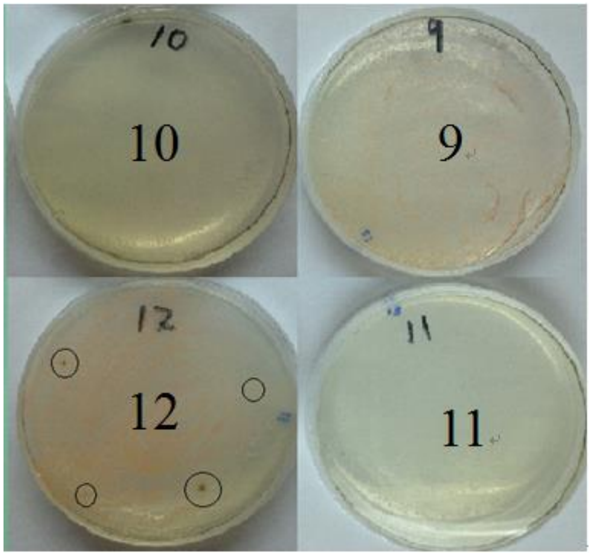

Fig. 7. The growth of bacterial colonies after an exposure time of one hour under the visible light irradiated.

Figure 8 shows photographs depicting the antibacterial effect of various films in the dark for 3 h. All Ag/TiO_2 films exhibit antibacterial activity after the prolonged exposure, with only the blank control sample (No. 19) showing several bacterial colonies.

Table. 4. Ag/TiO$_2$ ratios of various samples.

Samples No.	13	14	15	19
Ag： TiO2	1:9	3:7	1:1	none

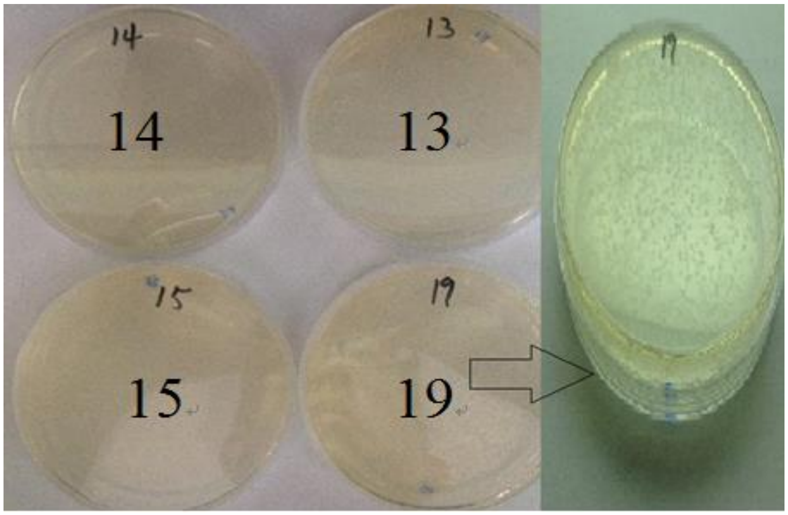

Fig. 8. The photograph of the antibacterial effect of various films under the dark condition for 3 hours.

4. Conclusions

1. Microwave synthesis can be used to rapidly prepare nanoscale silver particles 13–20 nm in size.
2. Under identical irradiation conditions, nanosilver particles exhibit higher antibacterial activity than do nano-TiO$_2$ thin films.
3. An increase in Ag content in Ag/TiO$_2$ films increases antibacterial activity regardless of the irradiation conditions.

References

Yeo, S.G., Ahn, C.H., & Kim, I.S. (2009). Polyethylene Glycol Applied in the Preparation of Ultrafine Silver Powder. *J. Korean Soc. Food Science*, *24*, 293–8.

Jung, B.O., Lee, Y.M., Kim, J.J., & Choi, Y.J. (2008). Nanometer Silver Preparation and Its Application THE. *Journal of Light Scattering*, *20*(1).

Liu, C.H., & Li, C.L. (2010). Research Progress of the Silver Nanoparticles Preparation Methods. *Chemical Research and Application*, *22*(6).

Zhang, W. (2009). Research and Development of Discoloration Inhibitor for Inorganic Antibacterial Agent Ag-Carrying. *New Chemical Materials*, *30*(4).

Feng, Q.L., Wu, J., Chen, G.Q., & Cui, F.Z. (2010). Synthesis of Silver NanoWires(Particles) in the Presence of Different Protective Agents. *J. Biomed. Mater. Res.*, *52*, 662–8.

Chen Y.H., Lin, A., & Gan, F.X. (2009). Preparation of Nano—Ti02 by Dialysis Hydrolysis of TICl4. *Journal of Inorganic Materials*, *22*(1).

Yang, X., Salzmann, C., & Green M.L.H. (2010). Preparation Characterization and Photocatalytic Activities of Novel Nanoscale Ag-TiO2 / ITO Films. *J. Phys. Chem. A*, *112*, 10784–10789.

Wang, D., Choi D., Li J., & Liu, J. (2009). Progress in Preparation and Application of Nano—titania. *ACS Nano*, *3*, 907–914.

Kim, C., Choi, M., & Jang, J. (2010). Optimization of the Preparation of Nano—Ti02 and Its Property. *Catal. Commun.*, *11*, 378–382.

Werts, M.P.L., Badila, M., & Brochon, C. (2008). Research and Development Of Discoloration Inhibitor

For Inorganic Antibacterial Agent. *Chem. Mater.*, *20*, 1292–1298.

Xu, M., Da, P., Wu, H., & Zhao, D. (2012). Preparation of Silver Nanoparticles via A Solvothermal Route. *Nano Lett.*, *12*, 1503–1508.

Jaiswal, S., McHale, P., & Duffy, B. (2012). Progress in Preparation of Nanosized Titanium Dioxide Photocatalyst. *Colloid Surf. B, 94,* 170–176.

Luu, C.L., Nguyen, Q.T., & Ho, S.T. (2010). Study of Antimicrobial Activity and Mechanism of Silver Nanoparticles on Escherichia Coli. *Adv. Nat. Sci.: Nanosci. Nanotechnol.*, *1*.

Stengl, V., Bakardjieva, S., & Murafa, N. (2003). Preparation and Antibacterial Effects of Ag–SiO2 Thin Films by Sol–Gel Method. *Biomaterials, 24,* 4921–4928.

Verne, E., Di Nunzioa, S., & Bosetti, M. (2005). Surface Characterization of Silver-Doped Bioactive Glass. *Biomaterials, 26,* 5111–5119.

Akhavan, O. (2009). Lasting Antibacterial Activities of Ag–TiO2/Ag/a-TiO2 Nanocomposite Thin Film Photocatalysts under Solar Light Irradiation. *Journal of Colloid and Interface Science*, *336*, 117–124.

Jeon, H.J., Yi, S.C., & Oh, S.G. (2003). Preparation and Antibacterial Effects of Ag–SiO2 Thin Films by Sol–Gel Method. *Biomaterials*, *24,* 4921–4928.

Balamurugana, A., & Balossierb, G. (2008). An In Vitro Biological And Anti-Bacterial Study On A Sol–Gel,Derived Silver-Incorporated Bioglass System. *Dental Material*s, *24,* 1343–1351.

Rai, M., & Yadav, A. (2009). Silver Nanoparticles as a New Generation of Antimicrobials. *Biotechnology Advances*, *27,* 76–83.

Akhavan, O. (2009). Lasting Antibacterial Activities of Ag–TiO2/Ag/a-TiO2 Nanocomposite Thin Film Photocatalysts under Solar Light Irradiation. *Journal of Colloid and Interface Science*, *336,* 117–124.