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Research Article

Synthesis of Silver-Doped Titanium TiO_2 Powder-Coated Surfaces and Its Ability to Inactivate *Pseudomonas aeruginosa* and *Bacillus subtilis*

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Hard, nonporous environmental surfaces in daily life are now receiving due recognition for their role in reducing the spread of several nosocomial infections. In this work, we established the photokilling effects of 1% silver-doped titanium dioxide TiO_2. The nanoparticles synthesized by liquid impregnation method were characterized using X-ray diffraction (XRD), energy dispersive spectroscopy (EDS), and scanning electron microscopy (SEM). The Ag-TiO_2 nanoparticle coatings that have been applied on glass and venetian blind surfaces were effective in generating a loss of viability of two bacteria (*Pseudomonas aeruginosa* and *Bacillus subtilis*) after two hours of illumination under normal light in the visible spectrum. Such surfaces can be applicable to medical and other facilities where the potential for infection should be controlled.

1. Introduction

Airborne transmission of nosocomial pathogens is of clinical and public interest [1]. Microbial infections are a serious problem in hospitals of many countries [2]. In order to prevent the transmission of such airborne infectious diseases to and control the spread of several types of airborne pathogens, self-sanitizing environmental surfaces are receiving due recognition in public places and dwellings [3, 4]. In recent years, nanoscaled antibacterial materials as novel antimicrobial species have been observed as promising candidates for application owing to their high surface-to-volume ratio and their novel physical and chemical properties on the nanoscale level. Many kinds of nanometer-sized antibacterial materials such as TiO_2, ZnO, MgO, chitosan, calamine, copper, and silver have been reported [5–10].

Titanium dioxide (TiO_2), a metal oxide semiconductor, has been found to be one of the most effective photocatalysts due to its high efficiency and stability (Hassan et al., 2011). TiO_2 is white, inexpensive, and nontoxic [11]. It is one of the most widely used photocatalysts for disinfection [12, 13]. Since the discovery of the photocatalytic splitting of water on a TiO_2 electrode under ultraviolet (UV) light [14], a great deal of research efforts have been made on semiconductor-based photocatalysts on both energy conversion and environmental applications. Bacterial cultures in contact with TiO_2-Pt thin film irradiated with near-UV light had a significant reduction in the number of cultivable cells [15]. The photocatalytic property has been widely studied in a variety of microorganisms such as viruses, bacteria, fungi, and algae. The main advantages of photocatalytic method are operation under ambient temperature and pressure, high stability and the low cost of catalyst, completed mineralization without creating secondary pollution, and possibility of using solar light ([12, 16–22] dheaya et al., 2008, [23]).

The TiO_2 photocatalyst is investigated as inhibitory agent for bacterial growth. TiO_2 is used in form of powder, films, and nanocomposites with a strong UV light as well as visible light. Little research work has been done regarding coating of metal-doped and nondoped TiO_2 nanoparticles aqueous solutions on substrates [12, 15, 17, 19, 23]. Recently, nanosize (<100 nm) TiO_2 particles have attracted a lot of attention of many researchers [24]. These nanometer-size TiO_2 particles exhibit many special properties due to the fact that the

bandgap of the nanoparticles increases with the decrease of their size, and the small TiO_2 particles offer a very large surface area [25].

Conventional methods of manual disinfection with wiping are not effective in the longer term, cannot be standardized, and are time intensive and labor intensive. In addition, there are problems associated with the use of aggressive chemicals [26]. There certainly is an urgent need of "green" products and procedures that can serve as environmentally surface disinfectants.

Self-cleaning surfaces can become a reality because of photocatalytic coatings containing TiO_2 nanoparticles. These nanoparticles initiate photocatalysis, a process by which pollutants are broken down by exposure to the sun's ultraviolet rays [27].

Today's self-cleaning surfaces are made by applying a thin nanoparticles coating film, painting a nanoparticles coating on, or integrating nanoparticles into the surface layer of a substrate material. Several studies show that TiO_2 nanoparticles create nontoxic, environmentally friendly, and hygienic photocatalytic coating. It creates a semipermanent invisible coating on most surfaces to provide antibacterial, antimold, antifungal, UV protection, deodorizing, air purification, self-cleaning, and self-sanitizing functionality [20, 23]. TiO_2 nanoparticles can create a self-cleaning effect on glass and ceramic surfaces [27]. The hypothesis that coating on substrates with silver-doped TiO_2 nanoparticles will decrease bacterial colonization is promising.

In this paper, we demonstrate the photocatalytic and bactericidal activity of metal-doped TiO_2 and silver-doped titanium. TiO_2 nanoparticles in aqueous solution are prepared by liquid impregnation method and dispersed to form coatings that can inhibit microbial growth on various substrates. The nanoparticles were characterized by X-ray diffraction (XRD), energy dispersive spectroscopy (EDS), and scanning electron microscopy (SEM). The antibacterial effects of those nanoparticles suspension and coatings were investigated, using *Pseudomonas aeruginosa* (gram-negative) and *Bacillus Subtilis* (gram-positive) bacteria.

2. Experimental Procedure

2.1. Materials System. One percent of Ag-TiO_2 nanoparticles was prepared by liquid impregnation method. Titanium (IV) dioxide (Sigma-Aldrich Laborchemikalien) and silver nitrate (Merck) were used in the liquid impregnation process as sources of titanium and silver, respectively. Distilled water was used as solvent in the process. The water employed in all preparations was purified by a distilled. Two bacteria strains, *P. aeruginosa* (ATCC 27853) and *B. subtilis* (ATCC 1174), were purchased from Microbiologics, Inc., USA. Other materials for bacteria cultivation, such as agar, sodium chloride, and plastic and pyrex petri dishes were of Merck grade.

2.2. Synthesis of Ag-TiO_2 Nanoparticles

2.2.1. Preparation of Photocatalyst: Liquid Impregnation Method. In the liquid impregnation method [28] silver ion (Ag^+) doped on TiO_2 was prepared according to the following steps.

We prepare 80 g of TiO_2-Ag nanoparticles, 79.2 g of TiO_2 was added to 500 mL of deionized water. Then for preparation of silver-doped TiO_2 nanoparticles, 1.7 g of $AgNO_3$ for doping was added to TiO_2 suspension; the silver concentration was of 1% (mole ratio) versus TiO_2. The slurry was stirred well for 6 hours and allowed to rest for 24 h and then dried in an air oven at 100°C for 12 h. The dried solids were crushed to fine powder in an agate mortar and calcined at 400°C for 6 h in a muffle furnace. In this method the metal gets deposited on the surface of the photocatalyst [29].

2.2.2. Characterization of Ag-TiO_2 Nanoparticles. The crystal structure of the Ag-TiO_2 nanoparticles was analyzed by X-ray diffractometer (Theta-Theta, Store, Germany). XRD measurements were carried out at room temperature with CuKα radiation ($\lambda = 0.15478$ nm) at 60 keV and 15 mA.

The topography, chemical composition, crystalline structure and metal deposition effect of the TiO_2, were determined by using scanning electron microscopy (SEM). The SEM samples were previously sputter coated with a gold film. For identification of the elements present in the nanoparticles and for determination of its chemical composition, energy-dispersive spectroscopy (EDS) embedded within JEOL JSM 6490A was used.

2.3. Bacterial Cultures and Test of Antibacterial Activities in Solution Phase

2.3.1. Bacterial Cultures. Two types of bacteria, *P. aeruginosa* ATCC 27853, a gram-negative bacterium, and *B. subtilis* ATCC 1174, a gram-positive bacterium, were used as model bacteria in this study.

P. aeruginosa is increasingly recognized as an emerging opportunistic pathogen of clinical relevance, ([20]; Eldere, 2003), while *B. subtilis* is known to cause disease in severely immunocompromised patients, and it can be conversely used as probiotic in healthy individuals [30]. *B. subtilis* has also been implicated in several cases of food poisoning [31].

Liquid culture of *P. aeruginosa* and *B. Subtilis* (*P. aeruginosa* strain ATCC 27853) was grown aerobically in nutrient broth (NB) at 37°C for 16 hours. The density of the microbial cells in liquid cultures was estimated by optical density (OD) at 600 nm wavelength. The OD was chosen in a range of 0.8–1.0, which is the optimal optical density of the cells for conventional bacterial activity testing. The cell suspensions used for antibacterial activity were approximately 9×10^9 colony-forming units cfu/mL. The bacteria concentration was also determined by a viable count procedure on nutrient agar plates after serial dilutions of the culture in 0.85% saline solution.

2.3.2. Photocatalytic Reaction

(1) Bacterial Activity Test of Ag-TiO_2 Nanoparticles on P. aeruginosa and B. subtilis. The photocatalyst used in this study was Ag-TiO_2 with a surface of 25 m^2/g (Janz et al.,

2010) and a primary particle size of 28–50 nm, well below the cut-off range (100 nm) that defines such particles. In the photocatalytic experiments, the flask containing 16-hour old culture of *P. aeruginosa* was adjusted on a magnetic stirrer. The required concentration of Ag-TiO_2 nanoparticle (10 mg/mL) was weighed and applied to the *P. aeruginosa* culture. The Ag-TiO_2-*P. aeruginosa* culture slurry was placed on a magnetic stir plate with continuous stirring at 250 rpm and was illuminated with simple fluorescent light. Bacterial activity test of TiO_2 nanoparticles on *B. subtilis* was tested in a similar fashion.

(2) Bacteria Viability Assay. The loss of viability was examined by the viable count procedure. The Ag-TiO_2 nanoparticles and bacterial culture slurry were exposed to simple fluorescent light with continuous stirring. A *P. aeruginosa* culture without TiO_2 was illuminated as a control, and the reaction of the TiO_2-bacterial culture, in the dark, was also carried out. Samples were taken at 0, 10, 20, 40, 60, 90, and 120 min intervals for two hours. The viable count was performed on nutrient agar plates after serial dilutions of the sample in saline solution. All plates were incubated at 37°C for 24 hours. Similar viable culture and count procedures were performed for the *B. subtilis* (ATCC 1174).

2.4. Immobilization of Ag-TiO_2 Nanoparticles on Substrates. Immobilization was done over two kinds of substrates, pyrex glass petri dish and plastic venetian blinds. All petri dishes were etched with dilute hydrofluoric acid (20% v/v) for 24 hours and washed thoroughly with deionized water, making a rough surface for better contact of TiO_2 on the glass surface. To remove organic and inorganic materials from the surface of the both substrates, they were treated with acetone and distilled water and dried under atmospheric conditions. Immobilization of Ag-TiO_2 nanoparticles was done on both substrates using the following two coating methods.

2.4.1. Water-Based Coating. In this method, distilled water was used as solvent. Ag-TiO_2 slurry was prepared with 1.5 g of Ag-TiO_2 in 200 mL of deionized water, and the suspension was placed in an ultrasonic bath for 15 min for dissolution. Substrates, glass and plastic venetian blinds, were immersed in the resultant slurry of Ag-TiO_2 for one hour and then removed from the suspension and placed in an oven for 1.5 hour at 150°C. The substrates, pyrex petri dishes and plastic venetian blinds, were subsequently placed in a furnace for 2 hours at 500°C and 160°C, respectively. The coated substrates were thoroughly washed with double-distilled water to remove any free Ag-TiO_2 particles (Khataee, 2009).

2.4.2. Ethanol-Based Coating. In this method, ethanol was used as solvent. Five grams of Ag-TiO_2 nanoparticles were dissolved in 180 mL of 99% ethanol to form the base medium of the slurry. Then, dilute nitric acid was added to adjust the pH to 3.5, which is necessary for dispersion of Ag-TiO_2 powder. This was followed by sonication for 15 min in an ultrasonic bath. Both substrates were immersed in the Ag-TiO_2 suspension for 60 min followed by drying in air for 24 hours. Then, pyrex glass petri dish and plastic venetian blind were heated at 475°C and 160°C, respectively, for 1 hour. Heating allows the Ag-TiO_2 nanoparticles to adhere more strongly to the substrate. Afterwards, the coated substrates were washed in deionized water to remove the unattached Ag-TiO_2 particles from the substrates (Vaez, 2012).

2.4.3. Bacterial Decontamination Effect of Ag-TiO_2 Nanoparticles-Coated Substrates. The viability of *P. aeruginosa* and *B. subtilis* was performed over coated substrates. Coated and uncoated (control) substrates were kept in sterile fume hood, and bacterial culture was sprayed on the plates as evenly as possible. The substrates were transferred to laminar flow cabinet while covered and exposed to fluorescent light (tube light in laminar flow cabinet). The sample for bacterial count was taken from coated and uncoated substrates at 0, 10, 20, 40, 60, 90, and 120 min intervals for two hours. The sample was prepared by taking swab thoroughly from the whole plate. Microbial count from substrate surfaces was performed using standard method [32].

3. Results and Discussions

3.1. Characterization of Materials

3.1.1. Crystal Phase Composition of Ag-TiO_2 Nanoparticles. X-ray diffraction was used to investigate the crystal phase composition and the crystallite size of Ag-TiO_2 nanoparticles. XRD patterns show that the nanoparticles contain pure anatase phase (JCPDS Card number 73-1764). No rutile reflection was seen in XRD patterns. Usually, a heat treatment at approximately 400°C is required for the phase transition of TiO_2 from amorphous to anatase phase in the solid state. The average crystallite sizes of the anatase phase of metal-doped TiO_2 were in the range of 28 to 50 nm.The XRD patterns are shown in Figure 1.

3.1.2. SEM Observations. SEM was used for the direct observation of particle size and morphology of sample powders. Figure 2 shows the images of 1% silver-doped TiO_2 nanoparticles by JEOL JSM-6460 at 500 and 20,000 magnifications. SEM images of Ag-TiO_2 nanoparticles confirm the presence of porous, sponge-like structure of high roughness and complexity. Such structure indicates the high surface area which has been proven to be efficient for photocatalytic degradation purposes. Samples consisted of more fine particles but the surface morphology of all the silver-doped TiO_2 samples was different from each other. The SEM pictures show that the distribution of silver on the surface of TiO_2 is not uniform, and silver-doped TiO_2 catalyst contains irregular shaped particles which are the aggregation of tiny crystals. Most of the particles were spheroid or oblate spheroid and loosed, and macropore can be clearly seen in the SEM micrographs. These nanoparticles are aggregated into microsized particles. The aggregation of these nanoparticles is beneficial to their removal from aqueous environment after the treatment.

SEM image of Figure 3 shows Ag-TiO_2 nanoparticles-coated glass surfaces have rough surface. Coating has formed

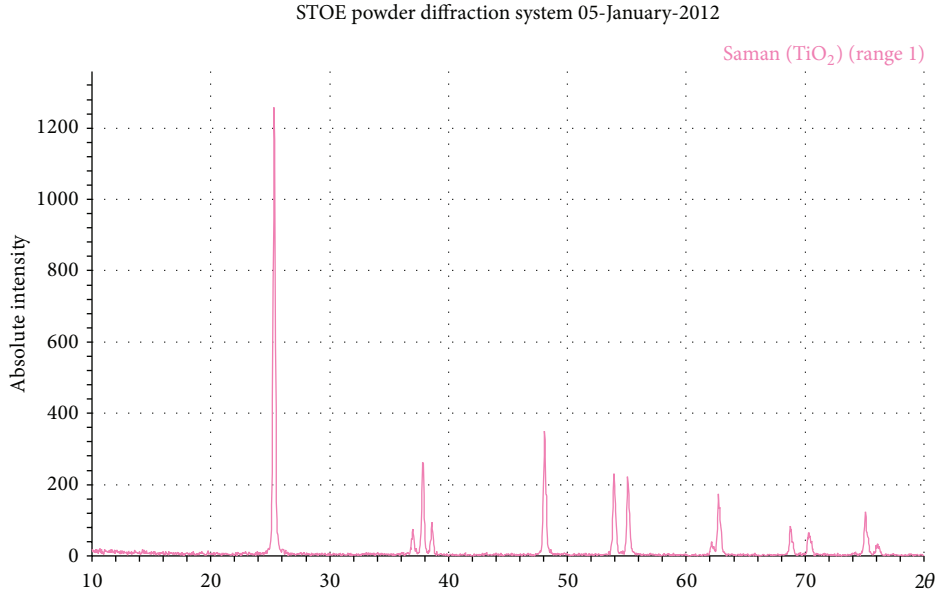

Figure 1: XRD patterns of metal-doped TiO_2 nanoparticles.

thick layer over surface of glass. Due to the high surface coverage of nanoparticles on the surface of the glass, it provides region for photodecomposition activity to act as a good self-sanitizing layer. Titania itself is not decomposed or being used in the process of disinfection. So weathering of layer is only limiting factor for that coating. Thick layer of nanoparticles over coated glass surface is advantageous as it is less affected to chance of weathering of coating as it consumes more time to get removed from surface so it has more life time.

SEM image of Figure 2(c) shows that nanoparticles get embedded into the plastic and fabric stands of plastic venetian blinds which are good surfaces for photocatalytic activity so that disinfection can be performed in a better manner.

3.1.3. Energy Dispersive Spectroscopy Analysis. EDS analysis showed that the percentage composition of Ag-TiO_2 nanoparticles was not consistent. The results indicated that titanium, oxygen, and silver were the constitutive elements of the nanoparticles prepared by the liquid impregnation method, and no extraneous elements were present (Figure 3). It also varied from point to point showing that the composition of the prepared nanoparticles was not homogenous, which confirmed the SEM results.

3.2. Loss of Viability of P. aeruginosa and B. subtilis under TiO_2 Photocatalytic Reaction by Ag-TiO_2 Nanoparticles

3.2.1. Photocatalytic Disinfection in Solution Phase. The antibacterial effect of nanoparticles on *P. aeruginosa* and *B. subtilis* was first tested for silver-doped TiO_2 solutions. The viability of Ag-TiO_2-treated bacteria cells was determined by colony counting after 24 h (*P. aeruginosa* and *B. subtilis*) of incubation. The viability of bacteria was significantly inhibited by the treatment of TiO_2 photocatalytic reaction. In the photocatalyzed Ag-TiO_2 nanoparticles, the survival of intact *P. aeruginosa* and *B. subtilis* colonies dropped in solution phase as a function of time as shown in Figure 3.

When the initial bacteria concentration was 9×10^9 cfu/mL, the survival of *P. aeruginosa* and *B. subtilis* colony dropped significantly after 30 min of photocatalytic reaction; bacteria killing was nearly complete within only 90 min under the present experimental conditions. A great decrease in the number of viable bacteria was observed on the illuminated TiO_2 nanoparticles, demonstrating their photokilling activity. The survival curve did not follow a simple single exponential decay process as a function of illumination time, but seemed to consist of two steps, a relative lower rate photokilling step, followed by a higher one (Figure 4). The result shows that there was no inhibition of bacterial growth in control solution. The bacterial load was reduced by 90% for *P. aeruginosa* and 90.5% for *B. subtilis* within our hour of treatment. The good antibacterial effect (100% killing efficiency) as observed in Figure 4 may be due to small size, large surface area, large bandgap energy, and more active sites of Ag-TiO_2 nanoparticles for carrying out photocatalytic reactions.

Anatase Ag-TiO_2 nanoparticles have shown bactericidal activities as they have inhibitory behavior to bacterial growth of *P. aeruginosa* (gram-negative bacteria) and *B. subtilis* (gram-positive bacteria) in the presence of light. This is attributed to the increasing visible absorption capacity due to the doping of silver in titanium nanoparticles [33]. The gram-positive bacteria have a relatively cell wall composed of many layers of peptidoglycan polymer and only one

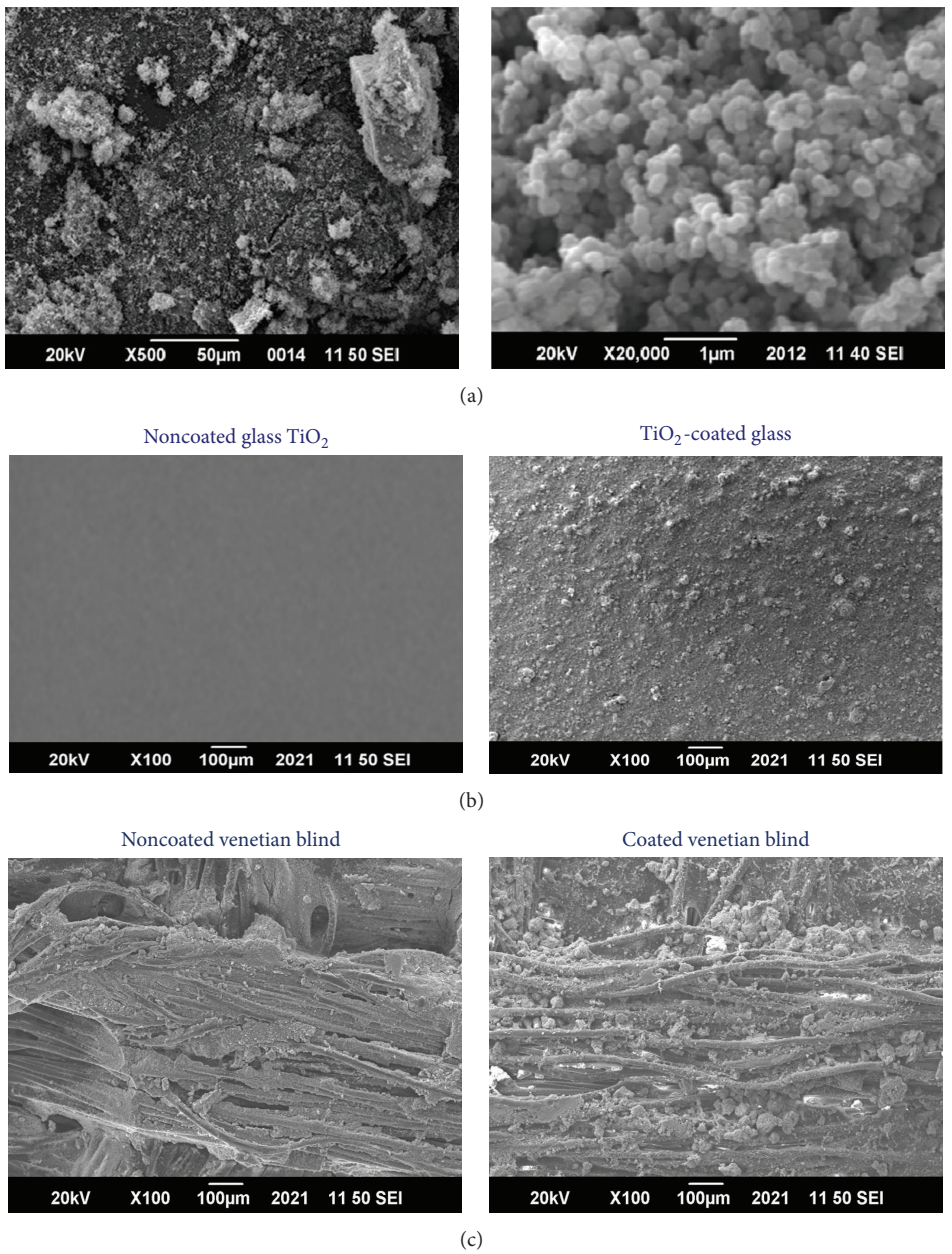

Figure 2: (a) SEM image of 1% Ag-doped TiO_2 taken at ×500 and at ×20,000. (b) SEM images of noncoated and TiO_2 nanoparticles-coated pyrex glass petri dish. (c) SEM images of non-coated and TiO_2 nanoparticles-coated plastic venetian blinds.

membrane (plasma membrane). The gram-negative bacteria have only a thin layer of peptidoglycan and more complex cell wall with two cell membranes, an outer membrane, and a plasma membrane. The addition of the outer membrane of the gram-negative bacteria cells influences the permeability of many molecules. Under certain conditions, the Gram-negative bacteria are more resistant to many chemical agents than Gram-positive cells [34].

The photocatalytic process of anatase Ag-TiO_2 nanoparticles includes chemical steps that produce highly reactive species such as hydroxyl radical, hydrogen peroxide, and superoxides that can cause grave damage to microorganisms. Among these reactive oxygen species, the hydroxyl radicals are highly reactive and therefore short lived. The superoxide ions are relatively longer lived. Due to their negative charge, they cannot penetrate the cell membrane. They must contact directly the outer surface of bacteria unless the TiO_2 particle has penetrated the cell. Hydrogen peroxide is less harmful compared to hydroxyl radicals and superoxide ions, but it can enter the cell [35].

Several proposed mechanisms for cell killing by the TiO_2 photocatalytic processes were reported [17, 18, 20, 36]. One research group reported direct evidence of cell membrane damage by the irradiation of a thin transparent TiO_2 film to

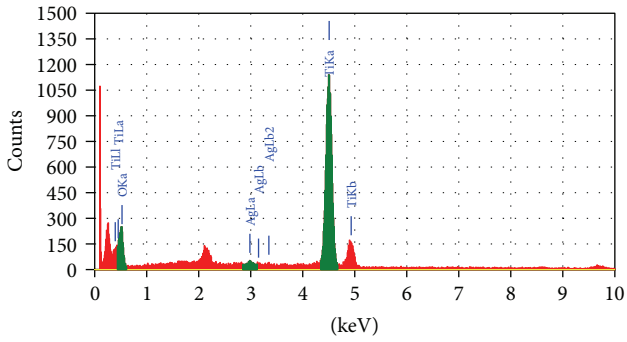

Figure 3: EDS pattern of Ag-TiO_2 nanoparticles prepared by liquid impregnation method.

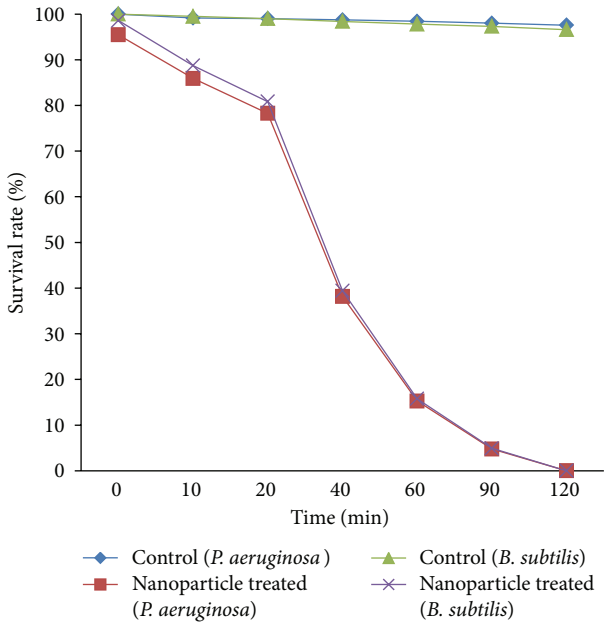

Figure 4: Survival rate of *P. aeruginosa* and *B. subtilis* cells in solution phase versus illumination time.

examine the photo-catalytic degradation of endotoxin from *P. aeruginosa*. The endotoxin is a component of the outer membrane of gram-negative bacteria and is released only when the cellular structure is destroyed. The results indicated that the TiO_2 photocatalyst destroys the outer membrane of the *P. aeruginosa* cell and causes the death of the bacteria, as damage of the cell membrane directly leads to the leakage of minerals, proteins, and genetic materials, causing cell death [15].

3.2.2. Bactericidal Effect of TiO_2 Nanoparticle-Coated Substrates. The survival rate of *P. aeruginosa* and *B. subtilis* on TiO_2 nanoparticle-coated substrates, pyrex glass petri dish, and venetian blinds, under photo-catalytic reaction is shown in Figures 5 and 6.

The bacterial colonies of *P. aeruginosa* and *B. subtilis* above Ag-TiO_2-coated glass plates and venetian blinds were significantly dropped on various substrates as a function of

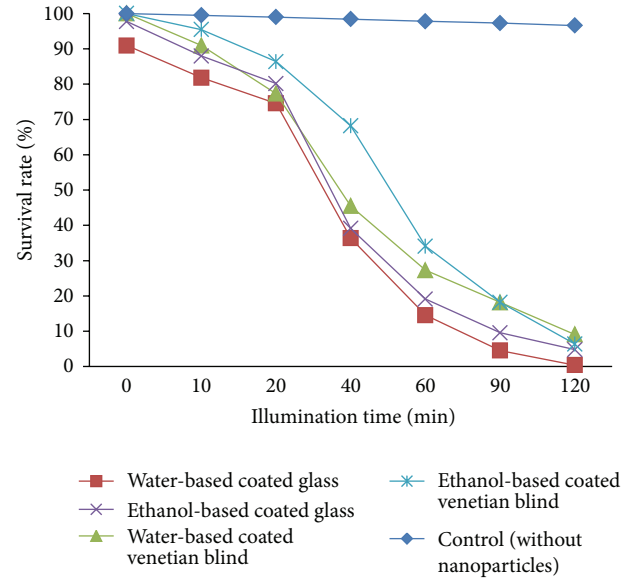

Figure 5: Survival rate of *P. aeruginosa* on different substrates as a function of time.

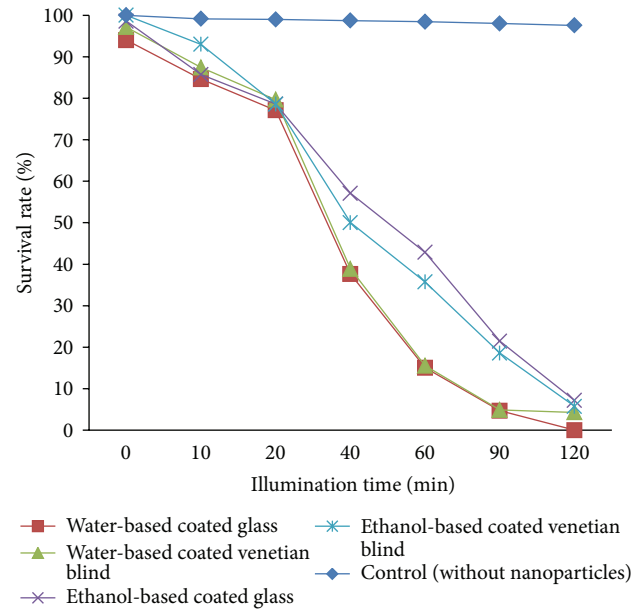

Figure 6: Survival rate of *B. subtilis* on different Ag-TiO_2 nanoparticle-coated substrates as a function of time.

time, while *P. aeruginosa* and *B. subtilis* proliferated well in the glass plate as well as venetian blinds which were not coated with Ag-TiO_2 nanoparticles. It was observed that the bacterial count decreases as a function of time. When the initial cell count of *P. aeruginosa* was 9×10^9 cfu/mL, the bacterial load was reduced by almost 85% on Ag-TiO_2 nanoparticles-coated glass petri dishes and 55% on Ag-TiO_2 nanoparticles-coated venetian blinds within ten minutes of exposure of light.

More than 90% decontamination was observed within 90 minutes exposure of light on Ag-TiO_2 nanoparticles-coated layer over substrates. Almost complete decontamination was

achieved within 120 minutes of treatment. Control samples showed no reduction in bacterial load as a function of time.

The decay of bacteria survival by the photokilling step was clearly demonstrated by bacteria viability assay. In control group of experiments, the number of bacterial count was above the countable range at the start of the experiment as well after 2 hrs. But a significant abatement in the bacterial count was observed within two hours: 0.4%, 9%, 4.7%, and 6.36% survival rates of *P. aeruginosa* on water-based coated glass, ethanol-based coated glass, water-based coated venetian blind, and ethanol-based coated venetian blind, respectively, and 0%, 4%, 7.1%, and 5.7% survival rates of *B. subtilis* on water-based coated glass, ethanol-based coated glass, water-based coated venetian blind, and ethanol-based coated venetian blind, respectively.

This illustrates that if 1% Ag-TiO_2 would be coated on a surface like glass and venetian blinds, that surface may be considered as self-sterilizing surface.

4. Conclusion

In this study, a novel and a simple liquid impregnation method was used for the synthesis of 1% silver doped anatase TiO_2 nanoparticles. The antibacterial test of those nanoparticles gave promising results which showed significant inhibition on both bacteria, *P. aeruginosa* and *B. subtilis*, even under room light.

Further testing of the survival ratio of *P. aeruginosa* and *B. subtilis* on the Ag-TiO_2 dispersed in coating formulations—coated glass and coated venetian blind—showed that Ag-TiO_2 nanoparticles coating over different substrates have 93%–100% killing efficiency towards bacteria. Ag-TiO_2 nanoparticles coatings when applied to substrate like glass or plastic venetian blinds disinfect the air that comes in contact with that substrate. So, ultimately these photocatalytic coatings containing silver-doped titanium dioxide (TiO_2) nanoparticles coating make self-sanitizing surfaces. Such Ag-TiO_2 coated surfaces should be employed in hospitals, public places, and dwellings to reduce the spread of infectious diseases.

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