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Antimicrobial Activity of Titanium Dioxide Nanoparticles Synthesized by Sol-Gel Technique

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Abstract: The process of Heterogeneous Photocatalysis (HP) using titanium dioxide photocatalysts is a field of immense research potential for researchers worldwide. TiO_2 as a photocatalyst has been widely applied for air and water remediation. This study reports the synthesis of a visible light responsive nanosized TiO_2 photocatalyst by a modified sol-gel process. The synthesized TiO_2 photocatalyst exhibits photocatalytic activity against some common pathogenic microorganisms such as *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *Staphylococcus aureus* under visible light illumination. TiO_2 is known to exhibit photocatalytic activity under UV light irradiation, the results obtained in this study using solar irradiation are very promising and enables the use of cheaply available solar energy for the process of photocatalysis.

Key words: TiO_2, sol-gel, photocatalysis, antimicrobial

INTRODUCTION

The emergence of new crop of alternative disinfection technologies (AOTs) such as Heterogeneous Photocatalysis (HP) and SODIS (Solar Disinfection) has been a very significant step in the direction of the concept of green technologies (Masakazu, 2000). Heterogeneous photocatalysis using titanium dioxide as a photocatalyst is an extensively studied area and has been applied in variety of fields such as air purification, wastewater treatment (Hur and Koh, 2002), photodegradation of toxic organic compounds, as disinfectant etc. (Huang *et al*., 1999; Maness *et al*., 1999; Vohra *et al*., 2005). The anatase type TiO_2 absorbs photons in the UV range of the solar spectrum exciting the valence electrons and generating the Electron-Hole Pairs (EHPs). These EHPs then recombine and become adsorbed on or near the surface of TiO_2. These excited electrons and holes have high redox activities and hence react with water and oxygen yielding Reactive Oxygen Species (ROS), such as super oxide anions (O_2^-) and hydroxyl radicals ($^{\bullet}OH$). The versatility of the photocatalysis process is complimented by the fact that the different $^{\bullet}OH$ radical production possibilities could be adapted for specific treatment requirements (Galvez *et al*., 2007). Ever since Matsunaga *et al*. (1988) reported for the first time the microbiocidal effect of TiO_2 photocatalytic reactions (Cho *et al*., 2005; Huang *et al*., 1999; Wong *et al*., 2006), the interest in this multifaceted compound has grown exponentially. Since then, research study on TiO_2 photocatalytic killing has been conducted intensively on a wide spectrum of organisms including bacteria (Huang *et al*., 1999; Maness *et al*., 1999; Tongpool *et al*., 2007), viruses (Cho *et al*., 2005; Gerrity *et al*., 2008; Lee *et al*., 1997), fungi (Mitoraj *et al*., 2007), cancer cells (Zhang and Sun, 2004), algal toxins (Srinivasan and Somasundaram, 2003).

Photocatalysis using TiO_2 has also been used to disinfect selective food-borne pathogens such as *Salmonella* sp. and *Listeria monocytogenes*, destroy *Bacillus anthracis* and the spores of *Bacillus subtilis* (Kau *et al*., 2009; Vohra *et al*., 2005). The main objectives of all these studies ranged from

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identifying the factors involved in photocatalytic disinfection, optimizing the conditions for the process, studying the mechanism and kinetics of photocatalytic disinfection on field applications (Cho *et al*., 2005; Sunada *et al*., 1998). The major challenge however has been to improve the photocatalytic efficiency of the process (Egerton *et al*., 2005).

Reactive oxygen species generated on irradiated TiO_2 surfaces, have been shown to operate in concert to attack polyunsaturated phospholipids in bacteria (Wong *et al*., 2006) and to catalyze site-specific DNA damage by generating H_2O_2 (Hirakawa *et al*., 2004) which might therefore result in subsequent cell death. TiO_2-mediated photooxidations have emerged as a promising technology for the elimination of microorganisms in many applications, e.g., self-cleaning and self-sterilizing materials (Cho *et al*., 2005).

The TiO_2 photocatalytic process is a conceptually feasible technology, however, since the UV region occupies only near 4% of the entire solar spectrum and 45% of the energy belongs to visible light (Yao *et al*., 2006), the potential applications of this promising technology are limited. The development of a visible-light responsive photocatalyst is therefore the need of the day.

In this study, we report the synthesis of sunlight responsive TiO_2 nanoparticles by a simple sol-gel method. The antibacterial activity of these TiO_2 nanoparticles was tested against four human pathogens *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *Staphylococcus aureus*.

MATERIALS AND METHODS

Preparation of TiO_2 Nanoparticles

Titanium (IV) chloride (Merck, 99%), was used as a precursor and was hydrolyzed under controlled conditions with continuous and rigorous stirring. The resulting sol was dialysed and the gel so obtained was dried in an oven at 100°C. The resultant white crystalline powder was calcined at 500°C and subsequently used for the study.

A comparative study was done using a commercial TiO_2 (Hombikat, Fluka, Sigma-Aldrich Chemie GmbH; Anatase).

Characterisation of TiO_2 Nanoparticles

XRD measurements of the TiO_2 nanoparticles were taken on a Rigaku Miniflex, operated at a voltage of 30 kV and a current of 15 mA using a Cu-Kα radiation of wavelength 1.5408 Å. The crystallite size was calculated using Scherer's equation, $D = k\lambda / B \cos\theta$; where, D is the crystallite diameter in Å, k is the shape constant (0.9), λ is the X-ray (Cu Kα) wavelength in Å , θ the diffraction angle and B (in radian) is the half width measured for the XRD peak.

Bacterial Cultures

All the bacterial strains *Escherichia coli* DH5α , *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *Staphylococcus aureus* were enriched in nutrient broth at 37°C for 18 h on a rotary shaker at 150 rpm. The enriched culture of each of the organisms was then serially diluted (10 fold) in sterile normal saline and used for the study.

Preparation of TiO_2 Suspension

A 0.1 M suspension of TiO_2 nanoparticles was prepared in sterile distilled water. The suspension was thoroughly mixed by vortexing. The suspension was always prepared fresh immediately prior to photocatalytic reaction and kept in the dark.

Photocatalytic Reaction

All the solutions and reagents were prepared with deionized and distilled water and analytical grade reagents were used throughout. All the glasswares and accessories used were washed with distilled water and then autoclaved at 121°C for 15 min. The photocatalytic reaction was carried out in a 100 mL glass beaker containing the bacterial cell suspension to which the uniformly mixed 0.1 M TiO_2 was added. The TiO_2-cell suspension was placed on a magnetic stir plate for continuous stirring so as to prevent the precipitation of TiO_2 nanoparticles and to ensure proper nanoparticle-cell contact. The suspension was illuminated with sun light.

Cell Viability Assay

The loss of viability of bacterial cells was monitored by taking the viable count after time intervals of 10, 20, 30 and 40 min. The TiO_2 cell suspension was exposed to visible light with continuous stirring. A control without TiO_2 was maintained. Aliquots of the TiO_2 cell suspension were withdrawn, serially diluted with saline and spread plated in duplicates on nutrient agar plates. All the plates were incubated at 37°C for 24 h.

RESULTS AND DISCUSSION

TiO_2 photocatalysts on irradiation with light of suitable wavelength causes the formation of Reactive Oxygen Species (ROS) which initiate a cascade of redox reactions which can mineralize a variety of organic compounds (Hirakawa *et al*., 2004; Lee *et al*., 1997). TiO_2 can have three types of crystal structures, anatase type, rutile type and brookite type. Among these, anatase type is known to have a higher photocatalytic activity because of the difference in the position of the conduction band (Three Bond, Technical news, 2004). Polycrystalline TiO_2 was synthesized by the sol-gel technique. The XRD pattern of the powder exhibited all the peaks corresponding to anatase phase of TiO_2 (Fig. 1) (Peng *et al*., 2005). The crystallite size of the TiO_2 was calculated to be ~13 nm using the Debye-Scherrer equation.

The bactericidal activity of these TiO_2 nanoparticles was determined by adding 0.1 M TiO_2 to the bacterial cell suspensions and exposing them to sunlight. The samples were withdrawn at regular time intervals and the number of surviving cells determined by viable count method. Figure 2-5 show the kill curves for the four test organisms *viz. Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *Staphylococcus aureus.* From an initial count of 10^5 cfu mL^{-1} a 2 log reduction (99%) in viable count could be obtained after 20 min of exposure in case of *Klebsiella pneumoniae* and *Pseudomonas aeruginosa,* whereas for *Staphylococcus aureus* a 1 log reduction (90%) was obtained after exposure for the same interval of time. At the end of 40 min a 5 log reduction (99.999%) was

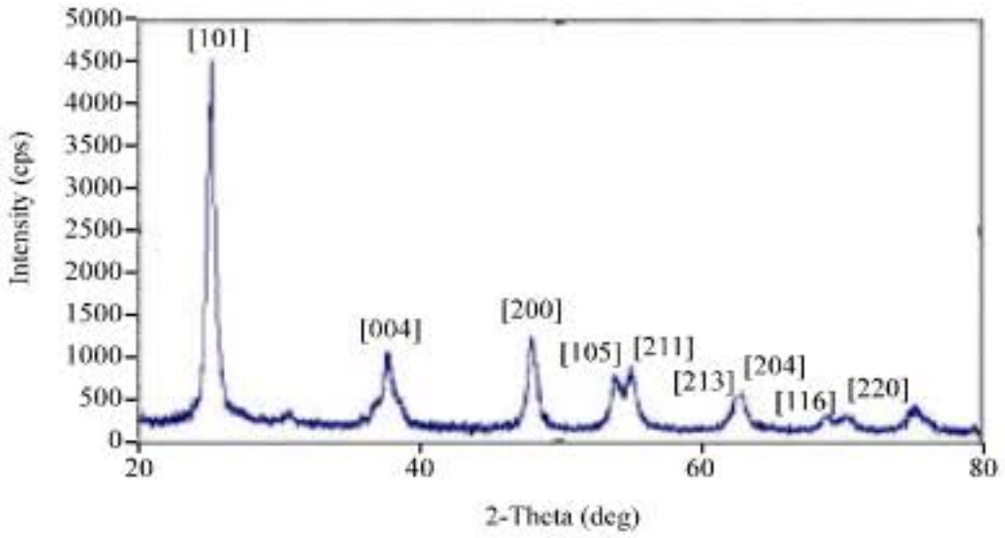

Fig. 1: XRD pattern of TiO_2 nanoparticles synthesized by sol-gel process

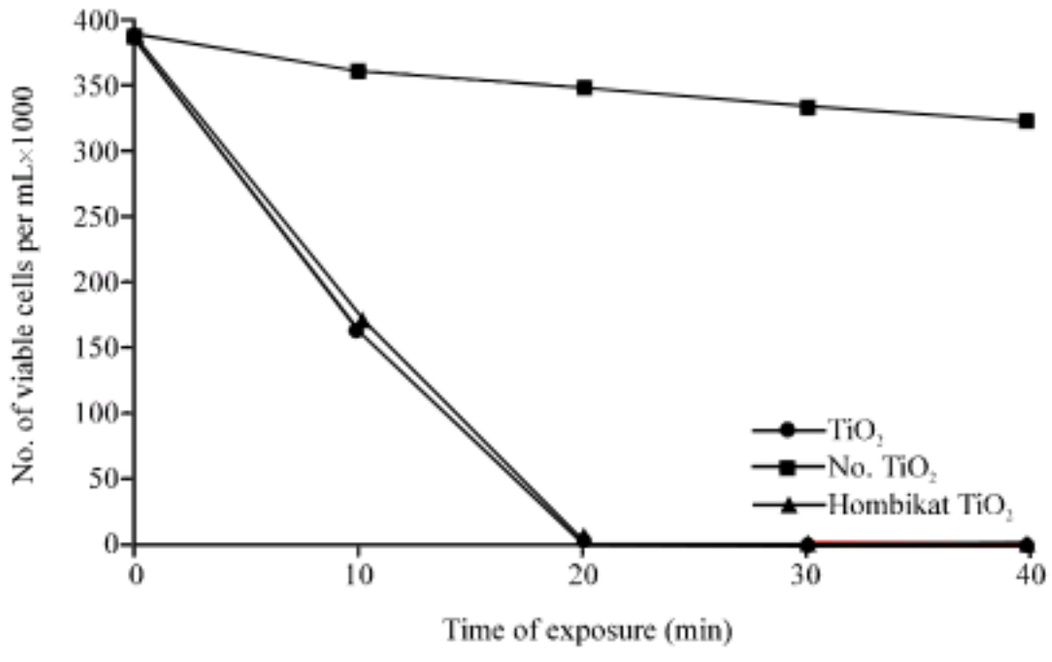

Fig. 2: Antibacterial activity of nanosized TiO_2 towards *Klebsiella pneumoniae*

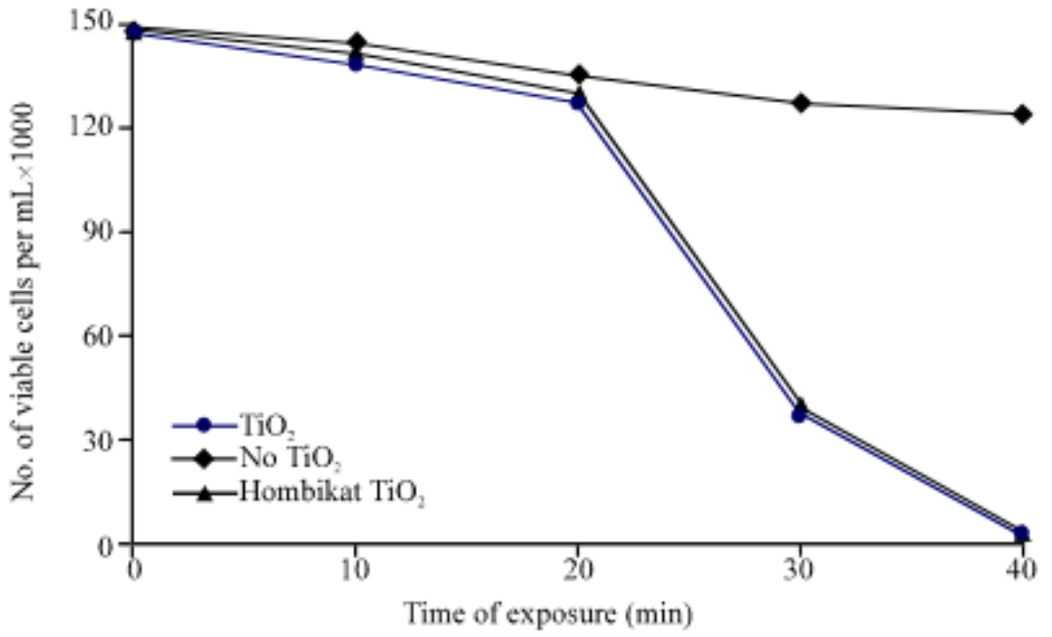

Fig. 3: Antibacterial activity of nanosized TiO_2 towards *E. coli*

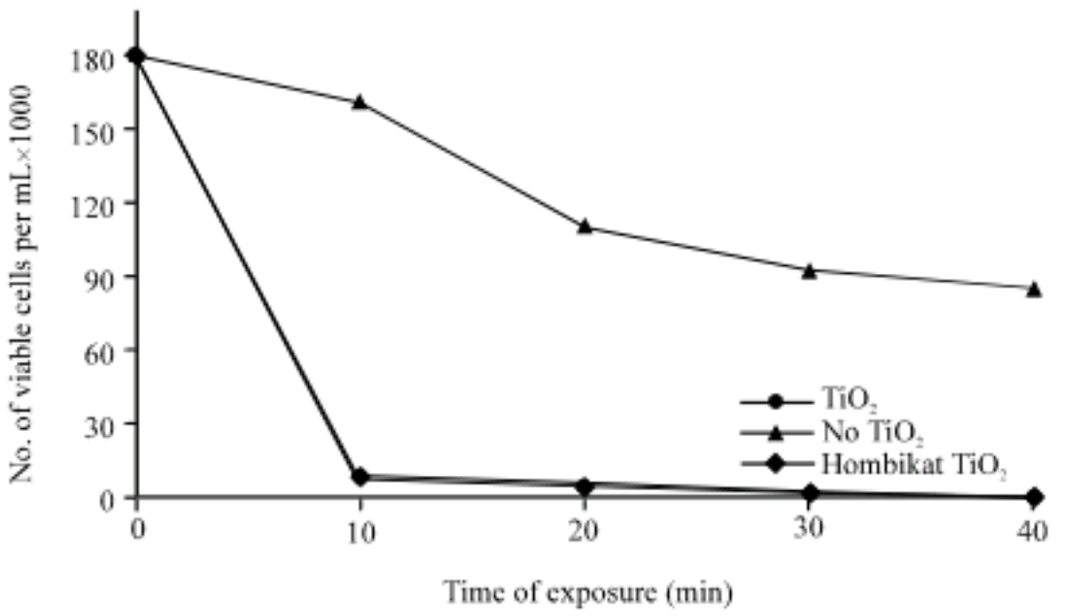

Fig. 4: Antibacterial activity of nanosized TiO_2 towards *Pseudomonas aeruginosa*

obtained for *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Staphylococcus aureus.* We observed that *Escherichia coli* exhibited minimal susceptibility to photocatalytic inactivation and a two

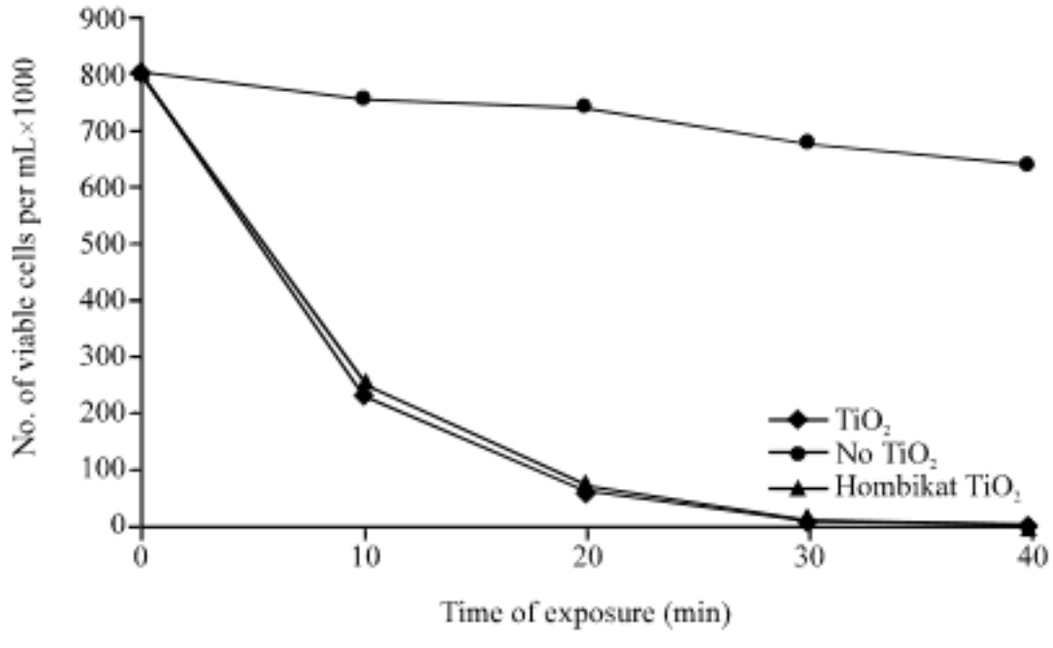

Fig. 5: Antibacterial activity of nanosized TiO_2 towards *staphylococcus aureus*

log reduction could be obtained, only after 40 min of exposure. Similar results for bactericidal activity were obtained with commercially available photocatalytic TiO_2 (Hombikat). The order of susceptibility of the organisms to inactivation by TiO_2 was *Staphylococcus aureus* > *Klebsiella pneumoniae*>*Pseudomonas aeruginosa*>*Escherichia coli*.

In all these experiments we observed that the efficiency of the process was reduced when the bacterial suspensions were not stirred continuously on a magnetic stirrer. To evaluate this we exposed the bacterial suspension + TiO_2 to sunlight and manually stirred the suspension once every 2 min. A 3 log reduction in viable count was obtained in the manually stirred beakers compared to the 5 log reduction that was observed in beakers stirred using the magnetic stirrer after 40 min of exposure to sunlight. Thus, the bactericidal action of TiO_2 was dependent on the amount of dissolved molecular oxygen and proper cell-TiO_2 contact both of which are increased during stirring. It has been reported earlier that, since TiO_2 photoreacts with oxygen present in O_2 and H_2O, more dissolved oxygen produce more $^{\bullet}OH$ radicals by scavenging the conduction band electrons and reducing the rate of EHP recombination. Thus, the lack of sufficient oxygen reduces the rate of reaction (Cho *et al*., 2004). In addition, free movement of the nanoparticles in suspension form facilitates the proper contact with microbes and accelerates the translocation of nanoparticles through the bacterial cell membrane (Williams *et al*., 2006).

A closer analysis of the survival curves of the microorganisms revealed that the inactivation had occurred in two distinct phases, the microorganisms were not much affected in the first 10 min. This could be due to the presence of $^{\bullet}OH$ radicals scavengers in water that can react with $^{\bullet}OH$ radicals generated by TiO_2 and reduce the efficiency of the inactivation process (Egerton *et al*., 2005). Studies on the mechanism of bactericidal activity of TiO_2 suggest that oxidative damage first takes place on the cell wall when the TiO_2 makes contact with the cell. Such cells are still viable, however, as photocatalytic action progresses the cell permeability increases, TiO_2 particles have easier access and cause photooxidation of intracellular components thereby accelerating cell death (Galvez *et al*., 2007). This further explains the initial delay in the bactericidal activity of TiO_2 nanoparticles.

The results of the antimicrobial study complies with the earlier findings by Matsunaga *et al*. (1988) and other researchers that irradiated TiO_2 exhibits bactericidal activity (Galvez *et al*., 2007; Vohra *et al*., 2005) and the efficacy of this disinfection process is proportionally correlated with the TiO_2 dose and the time of exposure. An approach of this kind, to solar disinfection of water using cost effective and reusable photocatalyst such as TiO_2 is very promising. The potential of this multifaceted compound for disinfection in environmental and medical fields is immense and further research needs to be done to harness its exceptional properties.

CONCLUSION

With the recent changes in the environmental scenario and emergence of newer environmental problems, simple and environment friendly technologies such as photocatalysis have attracted worldwide attention. Anatase titanium dioxide nanoparticles have been synthesized by a simple sol-gel technique. These nanoparticles have a crystallite size of ~13 nm and exhibit photocatalytic activity in presence of sunlight. TiO_2 is a known photocatalyst under UV light irradiation. The TiO_2 synthesized by sol-gel method exhibits bactericidal activity when irradiated with sunlight against the common human pathogens tested. This opens up newer avenues for the development of solar assisted alternative technologies for disinfection of water bodies contaminated with pathogenic organisms.

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