

Bactericidal Effects and Mechanisms of Visible Light-Responsive Titanium Dioxide Photocatalysts on Pathogenic Bacteria

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Abstract This review focuses on the antibacterial activities of visible light-responsive titanium dioxide (TiO₂) photocatalysts. These photocatalysts have a range of applications including disinfection, air and water cleaning, deodorization, and pollution and environmental control. Titanium dioxide is a chemically stable and inert material, and can continuously exert antimicrobial effects when illuminated. The energy source could be solar light; therefore, TiO₂ photocatalysts are also useful in remote areas where electricity is insufficient. However, because of its large band gap for excitation, only biohazardous ultraviolet (UV) light irradiation can excite TiO₂, which limits its application in the living environment. To extend its application, impurity doping, through metal coating and controlled calcination, has successfully modified the substrates of TiO₂ to expand its absorption wavelengths to the visible light region. Previous studies have investigated the antibacterial abilities of visible light-responsive photocatalysts using the model bacteria *Escherichia coli* and human

pathogens. The modified TiO₂ photocatalysts significantly reduced the numbers of surviving bacterial cells in response to visible light illumination. They also significantly reduced the activity of bacterial endospores; reducing their toxicity while retaining their germinating abilities. It is suggested that the photocatalytic killing mechanism initially damages the surfaces weak points of the bacterial cells, before totally breakage of the cell membranes. The internal bacterial components then leak from the cells through the damaged sites. Finally, the photocatalytic reaction oxidizes the cell debris. In summary, visible light-responsive TiO₂ photocatalysts are more convenient than the traditional UV light-responsive TiO₂ photocatalysts because they do not require harmful UV light irradiation to function. These photocatalysts, thus, provide a promising and feasible approach for disinfection of pathogenic bacteria; facilitating the prevention of infectious diseases.

Keywords Photocatalysis · Titania · Bactericidal effect · Visible light responsive · Bacterial spore · Mode of action

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Abbreviations

ROS Reactive oxygen species
UV Ultraviolet
AFM Atomic force microscopy
TiO₂ Titanium dioxide
O₂⁻ Superoxide anions
H₂O₂ Hydrogen peroxide

Introduction

Disinfection is one of the most common and important methods of controlling numbers of pathogens for the sterilization of critical instruments, water treatment, food

production, and in hospitals or health care facilities. Traditional chemical-based disinfectants, such as alcohols, aldehydes, iodine, phenols, and chlorine, have been used for centuries in environmental cleaning. Although these disinfectants are highly efficient against pathogenic microbes, they have drawbacks. Many of these disinfectants are volatile, and their byproducts can be toxic and carcinogenic to humans. The establishment and development of novel disinfection strategies is, therefore, significant in the control of human pathogens and prevention of infectious diseases.

Since the discovery of the photocatalytic water-splitting ability of titanium dioxide (TiO_2) electrodes in 1972 (Fujishima and Honda 1972), investigators have conducted extensive studies to understand, and to develop applications for, this material. One of the research interests was related to its possible application in energy renewal and storage (Bard 1982; Nazeeruddin et al. 2003; O'regan and Gratzel 1991). Another research area was application in environmental cleaning. In previous studies, treating polluted air and water with TiO_2 -based photocatalysts achieved total destruction of a large number of organic and inorganic compounds (Ho et al. 2006; Ranjit et al. 2001; Wong et al. 2006). Recently, research has extended to the potential application of TiO_2 -based photocatalysts in the treatment of pathogenic microbes, including bacteria, fungi, and viruses (Chen et al. 2009; Ditta et al. 2008; Foster et al. 2011; Maness et al. 1999; Cushnie et al. 2009; Yao et al. 2008); thus providing a new approach to disinfection.

Traditional pure TiO_2 photocatalysts are ultraviolet (UV) light-responsive (Li et al. 2008; Li and Zeng 2011; Rehman et al. 2009; Yang et al. 2011). Figure 1a shows a schematic diagram of the TiO_2 photocatalytic reaction. In a photocatalysis system, the electron of the photocatalyst becomes excited under UV irradiation from sunlight or an artificial light source. The excess energy of this excited electron promotes the electron to the conduction band of TiO_2 , creating a pair of a negatively charged free electron and a positively charged electron hole (electron vacancy in valence band; usually referred to as a hole or expressed as h^+ in Fig. 1). Identical processes occur in a photon-excited solar cell, for which the TiO_2 substrates were originally developed. The electrons and holes generated by the reactions have strong reducing and oxidizing activities, and subsequently react with atmospheric water and oxygen (H_2O and O_2) to yield reactive oxygen species (ROS), such as hydroxyl radicals ($\cdot\text{OH}$), superoxide anions (O_2^-), and hydrogen peroxide (H_2O_2). It is believed that the $\cdot\text{OH}$ radicals arise from hole trapping by surface hydroxyl groups, with H_2O being the primary oxidizing agent, and oxygen being a scavenger for photogenerated electrons to form ROS. However, it is not definitively known whether H_2O or O_2 is more extensively incorporated into the photooxidation

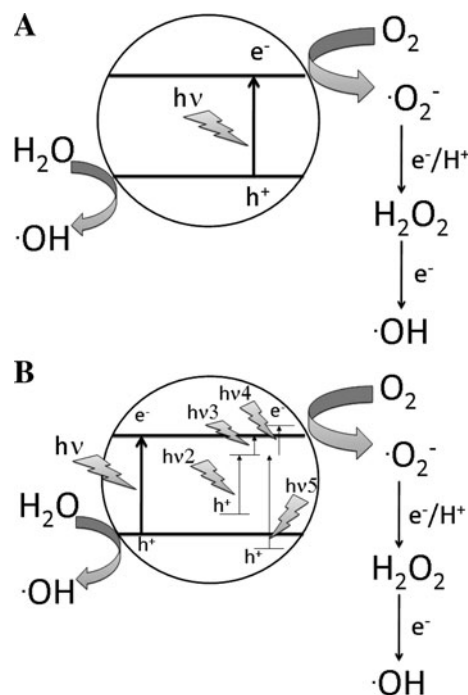


Fig. 1 Schematic diagram of the TiO_2 photocatalytic reaction. **a** The ultraviolet (UV)-responsive TiO_2 photocatalytic reaction. Owing to the large band gap, only high energy photons (UV, $h\nu$) can excite the TiO_2 to generate pair of free electrons (e^-) and an electron hole (h^+). **b** Following impurity doping, the photocatalysts remain excitable by UV photons, because of intraband gaps created by the impurity. However, lower energy photons, such as visible light ($h\nu_2$, $h\nu_3$, $h\nu_4$, and $h\nu_5$) can also excite the photocatalyst electrons in multiple steps

intermediates or products (Linsebigler et al. 1995). Holes and ROS are both extremely reactive when in contact with organic compounds; therefore, the pollutants in air and water can be eliminated when they come into contact with the surfaces of a photocatalyst. After a cycle of the photocatalytic reaction, the photocatalyst returns to its original state and is ready for another excitation. The photocatalytic reaction can, thus, continually eliminate organic and inorganic matter provided it has illumination. This also provides a promising strategy for the destruction of environmental pathogenic microbes. Previous studies achieved complete oxidation of organic compounds and *Escherichia coli* cells to carbon dioxide using photocatalysis (Jacoby et al. 1998; Maness et al. 1999; Wolfrum et al. 2002). Another study identified that ROS generated on irradiated TiO_2 surfaces was able to attack polyunsaturated phospholipids in bacteria (Maness et al. 1999). Previous research has also demonstrated that photoirradiated TiO_2 catalyzed site-specific DNA damage by generating H_2O_2 (Hirakawa et al. 2004). These findings suggested that TiO_2 might exert similar antimicrobial effects to those of the disinfectant H_2O_2 (McDonnell and Russell 1999). The oxidation of bacterial cell components, such as lipids and DNA, might, therefore, result in subsequent cell death (Maness et al. 1999). The

widespread use of antibiotics and the emergence of more resistant and virulent strains of microorganisms (Aiello and Larson 2003; Arias and Murray 2009; Christakis and Fowler 2010; Mariam et al. 2011; Russell 2003) has created the urgent need for the development of alternative sterilization technologies. The TiO₂ photocatalytic process is a feasible approach for disinfection. Compared with traditional disinfectants, the TiO₂ photocatalyst is safe, nontoxic, and does not produce hazardous byproducts (Fujishima 2000; Gamage and Zhang 2010). It is capable of repeated use; therefore, its costs can be minimized. However, owing to the wide band gap of TiO₂, only irradiation by UV light (wave length <400 nm), which accounts for only a small proportion of solar light (3–5 %), can excite the TiO₂ photocatalyst. In addition, the high energy UV radiation can induce serious damage to human tissues and cells which greatly restricts the potential application of TiO₂ substrates in the living environment. The development of a new TiO₂ photocatalytic system with increased activities under visible light illumination is, therefore, a significant and challenging task. Recent research has identified the impurity-doped TiO₂-based photocatalysts, which function under visible and UV light irradiation, offering the potential to extend their application in photocatalytic disinfection.

Visible Light-Responsive TiO₂ Photocatalysts

Although there are other materials which exert high photocatalytic activity (Chang et al. 2007, 2009, 2012), research on visible light-responsive photocatalysts has predominantly focused on TiO₂-based materials. Impurity doping is the most common approach to expand the spectral response of the TiO₂ photocatalyst to the visible light range (Kisch and Macyk 2002). Impurity doping creates intraband gaps (Fig. 1b) so that the lower energy photons, such as in visible light, can excite the electrons of the photocatalysts in multiple steps to attain the excitation levels of UV illumination. Previous studies originally used metal elements, such as silver, platinum, iron, chromium, cobalt, molybdenum and vanadium (Ag, Pt, Fe, Cr, Co, Mo, and V) to tune the electronic structure and enhance the photocatalytic activity of TiO₂. Temporarily trapping the photoexcited electrons using these doped impurities, and inhibiting the charge recombination, could also achieve enhanced photocatalysis (Chen et al. 2012; Nagaveni et al. 2004; Pan et al. 2010; Wong et al. 2006; Wu et al. 2010b; Zhang et al. 1998; Zhu et al. 2004, 2006). Recent efforts to modify TiO₂ photocatalysts using nonmetals, such as boron, carbon, nitrogen, sulfur and fluorine (B, C, N, S, and F), efficiently extended the photoresponse from the UV to the visible light region (Asahi et al. 2001; Ding et al. 2011; Ho et al. 2006; Huang et al. 2008; Yu et al. 2005; Zhao et al. 2004). Further theoretical calculations

also suggested that anion doping of TiO₂ has considerable effects on band gap alteration (Asahi et al. 2001).

To reduce the costs for synthesis of doped TiO₂, C and N doping are favorable. Yang et al. (2004a, b) developed several vapor deposition methods for preparation of N-doped and C-doped visible light-responsive TiO₂ photocatalyst films on various substrates, including silicon, glass, and quartz coupons. The N-doped TiO₂ showed visible light absorption with the red absorption edges shifted to approximately 565 nm; the C-doped TiO₂ films showed visible light absorption with the red absorption edges shifted to approximately 425 nm. The prepared nano-scale C- and N-doped thin films showed higher efficiency for photodegradation of methylene blue under visible light (>400 nm) irradiation compared to a pure TiO₂ thin film. The crystallinities and compositions of photocatalysts are correlated to their hydrophilic properties and photocatalytic activities during methylene blue degradation (Yang et al. 2004a, b). A sol-gel process using tetrabutyl orthotitanate and ethanol combined acid catalysis and low temperature calcination has also successfully synthesized visible light-responsive TiO₂ substrates with a mixed crystal lattice of anatase, brookite, and rutile (Tseng et al. 2006).

Success in developing visible light-responsive TiO₂ photocatalysts provides opportunities for extensive application in the elimination of chemical compounds and pollutants, such as carbon monoxide (CO), ethanol, gaseous 2-propanol, acetaldehyde, and the oxides of nitrogen (NO_x), and the decomposition of dyes such as methylene blue (Cong et al. 2007). It also provides a promising and feasible strategy for bacterial disinfection in environments where UV irradiation is limited or avoided.

Visible Light-Responsive TiO₂-based Photocatalysts for Antibacterial Application

Matsunaga et al. 1985 first reported the bactericidal activity of a UV-responsive TiO₂ photocatalyst in (Matusunga 1985; 1988). This has since become one of the most researched aspects of TiO₂ photocatalysis. Foster et al. (2011) reviewed the photocatalytic disinfection of more than 60 bacterial species, including Gram-positive and Gram-negative bacteria, using a wide range of UV-responsive TiO₂ materials and substrates. Their findings on UV-responsive TiO₂ will not be repeated herein.

Several groups have conducted studies on the application of impurity-doped visible light-responsive TiO₂ photocatalysts in bacterial inactivation (Cheng et al. 2009, 2012; Hamal et al. 2010; Hu et al. 2006; Pan et al. 2010; Wong et al. 2006; Wu et al. 2010a, b; Yu et al. 2005). Studies have also investigated possible photocatalytic bactericidal effects against the model bacteria *E. coli* using silver-modified TiO₂ (Pan et al. 2010; Wu et al. 2010b), Ag/N-codoped TiO₂ (Wu et al.

Table 1 Visible light-responsive TiO₂ photocatalysts shown to be effective against bacteria

| Visible light-responsive TiO ₂ photocatalysts | Bacteria killed | Forms of photocatalysts in action | References |
|--|--|-----------------------------------|-------------------------|
| S-doped nanocrystalline TiO ₂ | <i>Micrococcus lylae</i> | Suspension | Yu et al. 2005 |
| N-doped TiO ₂ | <i>Shigella flexneri</i> <i>Listeria monocytogenes</i> <i>Vibrio parahaemolyticus</i> <i>Staphylococcus aureus</i> <i>Streptococcus pyogenes</i> <i>Acinetobacter baumannii</i> | Solid thin film | Wong et al. 2006 |
| Ag/AgBr/TiO ₂ | <i>Escherichia coli</i> | Suspension | Hu et al. 2006 |
| AgI/TiO ₂ | <i>Escherichia coli</i> <i>Staphylococcus aureus</i> | Suspension | Hu et al. 2007 |
| N-doped TiO ₂ | <i>Bacillus subtilis</i> <i>Bacillus subtilis spores</i> <i>Bacillus thuringiensis spores</i> <i>Bacillus cereus spores</i> <i>Bacillus anthracis spores</i> | Solid thin film | Kau et al. 2009 |
| C-doped TiO ₂ | <i>Staphylococcus aureus</i> <i>Shigella flexneri</i> <i>Acinetobacter baumannii</i> | Suspension | Cheng et al. 2009 |
| Ag/TiO ₂ montmorillonite supported | <i>Escherichia coli</i> | Suspension | Wu et al. 2010b |
| Ag coated N-doped TiO ₂ | <i>Escherichia coli</i> <i>Staphylococcus aureus</i> <i>Streptococcus pyogenes</i> <i>Acinetobacter baumannii</i> | Solid thin film | Wong et al. 2010 |
| Cu-doped TiO ₂ | <i>Escherichia coli</i> | Suspension | Karunakaran et al. 2010 |
| Ag, C, S codoped TiO ₂ | <i>Escherichia coli</i> <i>Bacillus subtilis spores</i> | Suspension | Hamal et al. 2010 |
| N, Ag codoped TiO ₂ | <i>Escherichia coli</i> | Suspension | Wu et al. 2010a |
| C-doped TiO ₂ | <i>Escherichia coli</i> | Suspension | Liou et al. 2011 |

2010a), Ag/C/S-codoped TiO₂ (Hamal et al. 2010), AgI/TiO₂ (Hu et al. 2007), and Ag/AgBr/TiO₂ visible light-responsive photocatalysts (Hu et al. 2006). Wong et al. (2006) reported using an N-doped visible light-responsive TiO₂ photocatalyst to reduce the numbers of several human pathogens including *Shigella flexneri*, *Listeria monocytogenes*, *Vibrio parahaemolyticus*, *Staphylococcus aureus*, *Streptococcus pyogenes*, and *Acinetobacter baumannii*. Cheng et al. (2009) investigated the antibacterial activity of the visible light-irradiated C-doped TiO₂ on human pathogens, including *Shigella flexneri*, *S. aureus*, and *A. baumannii*; observing that photocatalysis was effective against these strains. Among these microorganisms, *S. flexneri*, *L. monocytogenes*, and *V. parahaemolyticus* usually exist in contaminated water, plants, and sewage (Chiou et al. 2001; Lima 2001; Martino et al. 2005; Wong et al. 2000), and frequently cause outbreaks in regions with poor public health conditions. The *S. pyogenes* and *S. aureus* species are exotoxin producing pathogens which can

cause soft tissue infections, food borne disease, and toxic shock syndrome (Salyers and Whitt 1994). Multidrug-resistant *A. baumannii* causing nosocomial infections are rapid spreading and of great concern in public health (Navon-Venezia et al. 2005). The N-doped TiO₂ photocatalysts were also proven effective against *Bacillus subtilis*, *Bacillus thuringiensis*, *Bacillus cereus*, and *Bacillus anthracis* under visible light illumination (Kau et al. 2009). To improve the bactericidal performance of the visible light-responsive TiO₂ photocatalysts, a recent study synthesized silver nanostructure coated N- and C-doped TiO₂ photocatalysts, testing them against the human pathogens *S. pyogenes*, *S. aureus*, and multidrug-resistant *A. baumannii* isolated from hospitals (Wong et al. 2010).

Table 1 summarizes the antibacterial spectra of various impurity-doped TiO₂ photocatalysts. All these photocatalysts exert significant visible light-inducible bactericidal effects. Some of these materials are produced as thin films;

the others work as powders in solutions (Table 1). Li et al. (2008) have applied UV-responsive titania photocatalysts as a suspension in a slurry UV reactor, as a thin film coated on a reactor surface, and as a membrane filter. This suggests the potential use of the visible light-responsive TiO₂ photocatalysts in a variety of settings to reduce the transmission of pathogens in public environments. Although the optimal conditions of antimicrobial treatments using the visible light-responsive TiO₂ photocatalysts have yet to be fully established, evidence has confirmed their effectiveness against bacteria, including human pathogens.

Reduction of Bacterial Endospore Activity

To combat harsh environments or survive in poor nutrition conditions, several types of bacteria produce tough and temporarily dormant structures called endospores. Endospores can survive desiccation, high temperatures, extreme freezing, and conditions without nutrients (Setlow 2005). They are also resistant to γ -radiation, UV radiation, and chemical disinfectants (Setlow 2005). Antibacterial agents, which destroy vegetative cell walls, have minimal effects on endospores. Bacterial endospores have, therefore, long caused serious problems in public health, food industry, and medicine because they are so difficult to kill. Lee et al. (2005) first used photocatalysis to treat bacterial endospores. They identified that *B. cereus* endospores were resistant to treatment using a commercial TiO₂ photocatalyst, Degussa P25, but treatment with a nanocomposite, composed of TiO₂ and carbon nanotubes, and solar UV lamps inactivated 90 % of these spores. Application of metal doping in TiO₂ photocatalysis further improved the efficiency of the UV-activated photocatalytic sporocidal effects (Vohra et al. 2005).

For visible light-responsive photocatalytic inactivation of bacterial endospores, Hamal et al. (2010) synthesized an Ag, C, and S codoped TiO₂ substrate, excitable under visible light, which showed strong antimicrobial properties against *E. coli* cells and *B. subtilis* spores. Kau et al. (2009) reported that photocatalysis by visible light-responsive TiO₂ substrates doped with N and C significantly reduced the cell numbers and spore activity of *B. subtilis*, *B. thuringiensis*, *B. cereus*, and *B. anthracis*. In this report, findings on the photocatalytic treatment of the endospores of the bacterial strains were of particular interest. Although the visible light-induced photocatalysis did not kill the spores as effectively as the cells, injection of the photocatalysis-treated *B. anthracis* spores into mice greatly reduced their mortality (from 100 % mortality for untreated spores to 33 % mortality for photocatalyst-treated spores) (Kau et al. 2009). These results indicated that despite incomplete destruction of the endospores, the photocatalysis inactivated some of

their pathogenic components without eliminating their germination ability, thus reducing the induced mortality.

A Comparison between Photocatalysts and Antibiotics

Since the first application of photocatalysts in disinfection, investigators have conducted extensive research on the bactericidal mechanisms of photocatalysis. Kohanski et al. (2007) described that bactericidal antibiotics, including aminoglycosides, quinolone, and β -lactams, share a common mechanism for the killing of bacteria, despite targeting different components of different cells. These compounds all converge into a common hydroxyl radical production pathway which induces bacterial cell death (Kohanski et al. 2007). This suggests that a bacterium might become multidrug and photocatalysis resistant by finding a way to avoid the effects of the hydroxyl radical production. However, a comparative study of the bactericidal effects of TiO₂ against antibiotic-resistant and antibiotic-sensitive bacteria produced results to indicate otherwise (Tsai et al. 2010).

The previously mentioned study applied TiO₂ treatment, under UV-A illumination, to methicillin-resistant *S. aureus*, multidrug-resistant *A. baumannii*, and vancomycin-resistant *Enterococcus faecalis*, comparing the findings with those obtained using the same treatment on antibiotic-sensitive strains (Tsai et al. 2010). The results indicated that in the presence of UV-A, TiO₂ effectively reduced the number of antibiotic-resistant microbes in suspension. The methicillin-resistant and antibiotic-sensitive *S. aureus* strains were equally susceptible to TiO₂ photocatalysis. However, multidrug-resistant *A. baumannii* was more susceptible to TiO₂ photocatalysis than the antibiotic-sensitive strain. In contrast, vancomycin-resistant *E. faecalis* was less susceptible to the photocatalytic treatment than vancomycin-sensitive *E. faecalis*. Although the mechanisms for multidrug resistance have yet to be fully elucidated, these results indicate that antibiotic-resistant bacteria are not equally resistant to photocatalysis. This suggests that the use of photocatalysts could be complementary to existing disinfection technologies and, thus, facilitate the control of the spread of pathogenic bacteria.

Electron Microscopic Analyses

To elucidate the killing mechanism of visible light-responsive photocatalysis by modified TiO₂ substrates requires direct observation of bacterial cell death during treatment. This would normally occur on a nanometer scale; thus necessitating the use of high-resolution imaging techniques. Electron microscopy is often used. Studies have also reported scanning electron micrographs of visible

light-responsive TiO_2 photocatalysis of Gram-positive and methicillin-resistant *S. aureus* strains (Cheng et al. 2009; Tsai et al. 2010). These micrographs clearly demonstrated that photocatalysis altered the bacterial cell morphology. Bacterial cell disruption caused by UV-responsive TiO_2 (Tsai et al. 2010) and visible light-responsive TiO_2 (Cheng et al. 2009) were not distinguishable. Studies have also used scanning and transmission electron microscopy to visualize the destructive effects of visible light-responsive TiO_2 on *E. coli* cells (Hu et al. 2006, 2007; Wu et al. 2010a, b). In these images, the damaged bacterial membranes and leakage of bacterial components were clearly evident. Although some studies used electron microscopy to observe uneven damage to rod-shaped *E. coli* cells (Chamakura et al. 2011; Hu et al. 2007; Wu et al. 2010b), without careful statistical measurements, the killing mechanism remains unclear. The bacterial images in these studies were usually acquired after a treatment time of 30 min to 3 h. After these long treatment times, death of the majority of these cells had occurred, and most evidence regarding the bacterial killing mechanism had been lost.

Atomic Force Microscopic Analysis of Antibacterial Activity

Atomic force microscopy (AFM) is another technique for acquiring high resolution images of bacterial cells. Unlike electron microscopy, preparation of samples for AFM imaging does not require harsh physical and chemical treatments. This minimizes distortion of the samples during the imaging process. Figure 2 displays representative AFM images of *E. coli* cells before and after 1 min photocatalytic treatment using a C-doped visible light-responsive TiO_2 photocatalyst. Recently, our group used AFM to analyze the visible light-driven photocatalyst-mediated damage to *E. coli* (Liou et al. 2011); imaging *E. coli* cells following 1–5 min photocatalytic treatment. These short treatment times ensured that cell damage was in the initial stages. Results indicated that the antibacterial properties of visible light-responsive photocatalysis were associated with hole-like structures formed by the photocatalytic reactions. Cell destruction can be initiated at any position on the bacterial cells; however, statistical analysis identified that the bacterial cell damage tended to be preferentially induced at, or near, the poles of rod-shaped *E. coli* cells. This study also demonstrated that photocatalysis caused various levels of cell damage, and was likely to elicit damage in a sequential manner, in *E. coli* cells. Figure 3 displays a hypothetical mechanism for the photocatalytic bactericidal effects. The process began with changes to the surface properties of bacterial cells, as indicated by surface roughness measurements using AFM,

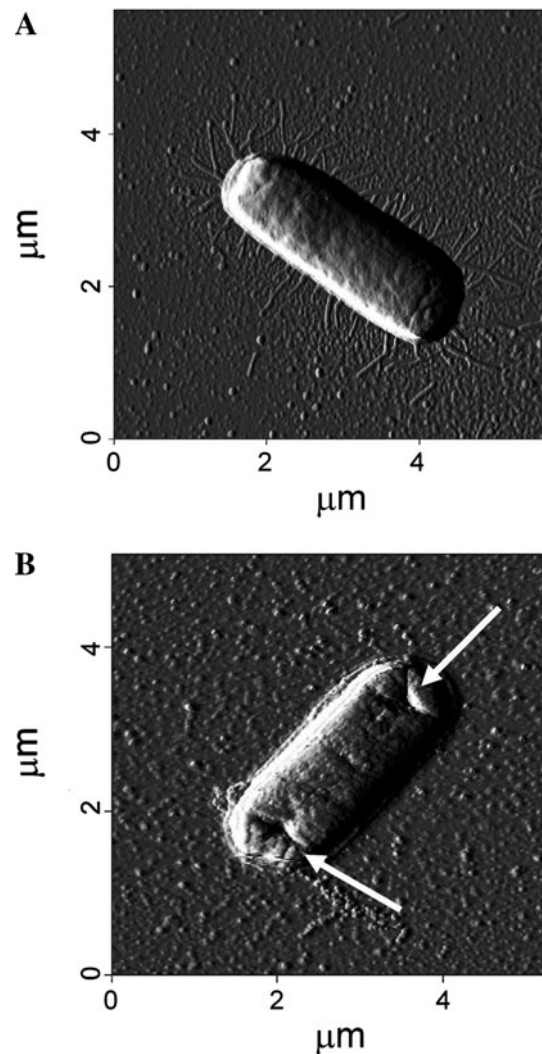


Fig. 2 Atomic force microscopic images of *E. coli* cells before (a) and after (b) 1 min photocatalytic treatment by a C-doped photocatalyst, C200. The white arrows indicate sites of damage on the bacterial surface caused by the photocatalysis

and holes formed at the poles of cells. The holes then enlarged until total transformation into a flattened shape. Experiments using UV light-responsive TiO_2 substrates produced similar findings (Liou et al. 2011), suggesting that this is a general *E. coli* cell response to photocatalysis.

Application of Visible Light-Responsive TiO_2 Photocatalysts in Disinfection

The application of photocatalysts in disinfection remains under development and in the early stages of commercialization. Photocatalytic disinfection could potentially reduce the use of chemical disinfectants. Commercial applications of photocatalyst products include air conditioning systems and restroom disinfections. Figure 4 shows

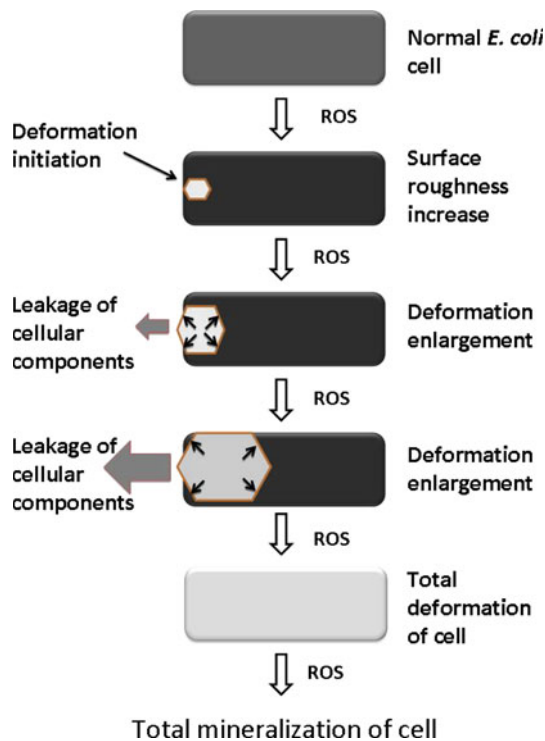


Fig. 3 Proposed bactericidal mechanism of ROS produced by TiO₂ photocatalysis



Fig. 4 An example of the application of a photocatalyst for antibacterial purposes. An official notice posted above a restroom door on a train belonging to the Taiwan Rail Administration (TRA), Taiwan. This notice indicates that photocatalysts disinfected the restroom

an example of the commercial application of a photocatalyst in restroom disinfection. Photocatalysts have also been used to treat polluted water (Gamage and Zhang 2010), and can be applied as coating materials for surface disinfections, or suspended in liquid and filled into columns for water/liquid cleaning. However, most commercial photocatalysts are excited by UV, which occupies a small proportion of sunlight. Additional UV illumination is required for indoor application, thus increasing the costs. The development of visible light-responsive photocatalysts

could potentially further extends the application of photocatalysts to include places where UV light is limited.

Conclusions

Disinfection is one of the most important and commonly used strategies to control numbers of pathogens. In contrast to chemical disinfectants, photocatalysts provide a relatively new approach for controlling the numbers of pathogenic bacteria. Titanium dioxide is one of the most commonly applied photocatalysts. The TiO₂ photocatalyst is safe, reusable, and does not produce hazardous byproducts. However, because of a large band gap for excitation, only high energy UV irradiation, which is harmful to humans and limited in indoor environments, can excite TiO₂. Its applications are, therefore limited. Impurity doping, through metal coating and controlled calcination, have successfully modified the substrates of TiO₂ to extend its absorption wavelengths to the visible light region. Previous studies have investigated the antibacterial activities of visible light photocatalysts on model bacteria *E. coli* and human pathogens. The modified TiO₂ photocatalysts significantly reduced the numbers of surviving bacterial cells in response to visible light irradiation. Photocatalysis can also significantly reduce the activity of bacterial endospores; reducing their toxicity while maintaining their germination abilities. During the photocatalytic killing mechanism, photocatalysis initially damages the surfaces of the bacterial cells, before breakage of the cell membranes occurs at weak points. Subsequently, the internal bacterial components leak from the cells through the damaged sites. Finally, the photocatalysis destroys the cell debris. As summarized, visible light-responsive TiO₂ photocatalysis provides a promising, feasible, and safe approach for disinfection of pathogenic bacteria; thus facilitating the prevention of microbe-related diseases.

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References

- Aiello AE, Larson E (2003) Antibacterial cleaning and hygiene products as an emerging risk factor for antibiotic resistance in the community. *Lancet Infect Dis* 3:501–506
- Arias CA, Murray BE (2009) Antibiotic-resistant bugs in the 21st century—a clinical super-challenge. *New Engl J Med* 360:439–443
- Asahi R, Morikawa T, Ohwaki T et al (2001) Visible-light photocatalysis in nitrogen-doped titanium oxides. *Science* 293:269–271

- Bard AJ (1982) Design of semiconductor photoelectrochemical systems for solar energy conversion. *J Phys Chem* 86:172–177
- Chamakura K, Perez-Ballesteros R, Luo Z et al (2011) Comparison of bactericidal activities of silver nanoparticles with common chemical disinfectants. *Colloids Surf B Biointerfaces* 84:88–96
- Chang WK, Rao K, Kuo HC et al (2007) A novel core-shell like composite $\text{In}_2\text{O}_3/\text{CaIn}_2\text{O}_4$ for efficient degradation of methylene blue by visible light. *Applied Catalysis A* 321:1–6
- Chang WK, Wub YS, Tzeng CY et al (2009) Photocatalytic degradation and properties of core-shell like composite $\text{In}_2\text{O}_3/\text{Ba}_2\text{In}_2\text{O}_5$ synthesized via chemical impregnation. *J Alloys Compounds* 478:341–344
- Chang WK, Sun DS, Chan H et al. (2012) Visible light responsive core-shell structured $\text{In}_2\text{O}_3/\text{CaIn}_2\text{O}_4$ photocatalyst with superior bactericidal property and biocompatibility. *Nanomedicine-NBM* 8
- Chen F, Yang X, Wu Q (2009) Photocatalytic oxidation of *Escherichia coli*, *Aspergillus niger*, and formaldehyde under different ultraviolet irradiation conditions. *Environ Sci Technol* 43:4606–4611
- Chen YL, Chen YS, Chan H et al (2012) The use of nanoscale visible light-responsive photocatalyst TiO_2 -Pt for the elimination of soil-borne pathogens. *PLoS One* 7:e31212
- Cheng CL, Sun DS, Chu WC et al (2009) The effects of the bacterial interaction with visible-light responsive titania photocatalyst on the bactericidal performance. *J Biomed Sci* 16:7
- Chiou CS, Hsu WB, Wei HL et al (2001) Molecular epidemiology of a *Shigella flexneri* outbreak in a mountainous township in Taiwan, Republic of China. *J Clin Microbiol* 39:1048–1056
- Christakis NA, Fowler JH (2010) Social network sensors for early detection of contagious outbreaks. *PLoS One* 5:e12948
- Cong Y, Zhang J, Chen F et al (2007) Preparation, photocatalytic activity, and mechanism of nano- TiO_2 co-doped with nitrogen and iron (III). *J Phys Chem C* 111:10618–10623
- Cushnie TP, Robertson PK, Officer S et al (2009) Variables to be considered when assessing the photocatalytic destruction of bacterial pathogens. *Chemosphere* 74:1374–1378
- Ding X, Song X, Li P et al (2011) Efficient visible light driven photocatalytic removal of NO with aerosol flow synthesized B, N-codoped TiO_2 hollow spheres. *J Hazard Mater* 190:604–612
- Ditta IB, Steele A, Liptrot C et al (2008) Photocatalytic antimicrobial activity of thin surface films of TiO_2 , CuO and TiO_2/CuO dual layers on *Escherichia coli* and bacteriophage T4. *Appl Microbiol Biotechnol* 79:127–133
- Foster HA, Ditta IB, Varghese S et al (2011) Photocatalytic disinfection using titanium dioxide: spectrum and mechanism of antimicrobial activity. *Appl Microbiol Biotechnol* 90:1847–1868
- Fujishima A (2000) Titanium dioxide photocatalysis. *J Photochem Photobiol C* 1:1–21
- Fujishima A, Honda K (1972) Electrochemical photolysis of water at a semiconductor electrode. *Nature* 238:37–38
- Gamage J, Zhang Z (2010) Applications of photocatalytic disinfection. *Intl J Photoenergy* 2010:764870
- Hamal DB, Haggstrom JA, Marchin GL et al (2010) A multifunctional biocide/sporicide and photocatalyst based on titanium dioxide (TiO_2) codoped with silver, carbon, and sulfur. *Langmuir* 26:2805–2810
- Hirakawa K, Mori M, Yoshida M et al (2004) Photo-irradiated titanium dioxide catalyzes site specific DNA damage via generation of hydrogen peroxide. *Free Radic Res* 38:439–447
- Ho W, Yu JC, Lee S (2006) Synthesis of hierarchical nanoporous F-doped TiO_2 spheres with visible light photocatalytic activity. *Chem Commun* 10:1115–1117
- Hu C, Lan Y, Qu J et al (2006) Ag/AgBr/ TiO_2 visible light photocatalyst for destruction of azodyes and bacteria. *J Phys Chem B* 110:4066–4072
- Hu C, Guo J, Qu J et al (2007) Photocatalytic degradation of pathogenic bacteria with AgI/ TiO_2 under visible light irradiation. *Langmuir* 23:4982–4987
- Huang Y, Ho W, Lee S et al (2008) Effect of carbon doping on the mesoporous structure of nanocrystalline titanium dioxide and its solar-light-driven photocatalytic degradation of NOx. *Langmuir* 24:3510–3516
- Jacoby WA, Maness PC, Wolfrum EJ et al (1998) Mineralization of bacterial cell mass on a photocatalytic surface in air. *Environ Sci Technol* 32:2650–2653
- Karunakaran C, Abiramasundari G, Gomathisankar P et al (2010) Cu-doped TiO_2 nanoparticles for photocatalytic disinfection of bacteria under visible light. *J Colloid Interface Sci* 352:68–74
- Kau JH, Sun DS, Huang HH et al (2009) Role of visible light-activated photocatalyst on the reduction of anthrax spore-induced mortality in mice. *PLoS One* 4:e4167
- Kisch H, Macyk W (2002) Visible-light photocatalysis by modified titania. *ChemPhysChem* 3:399–400
- Kohanski MA, Dwyer DJ, Hayete B et al (2007) A common mechanism of cellular death induced by bactericidal antibiotics. *Cell* 130:797–810
- Lee SH, Pumpreug S, Moudgil B et al (2005) Inactivation of bacterial endospores by photocatalytic nanocomposites. *Colloids Surf B Biointerfaces* 40:93–98
- Li W, Zeng T (2011) Preparation of TiO_2 anatase nanocrystals by TiCl_4 hydrolysis with additive H_2SO_4 . *PLoS One* 6:e21082
- Li Q, Mahendra S, Lyon DY et al (2008) Antimicrobial nanomaterials for water disinfection and microbial control: potential applications and implications. *Water Res* 42:4591–4602
- Lima AA (2001) Tropical diarrhoea: new developments in traveller's diarrhoea. *Curr Opin Infect Dis* 14:547–552
- Linsebigler AL, Lu G, Yates JT (1995) Photocatalysis on TiO_2 surfaces: principles, mechanisms, and selected results. *Chem Rev* 95:735–758
- Liou JW, Gu MH, Chen YK et al (2011) Visible light responsive photocatalyst induces progressive and apical-terminus preferential damages on *Escherichia coli* surfaces. *PLoS One* 6:e19982
- Maness PC, Smolinski S, Blake DM et al (1999) Bactericidal activity of photocatalytic TiO_2 reaction: toward an understanding of its killing mechanism. *Appl Environ Microbiol* 65:4094–4098
- Mariam SH, Werngren J, Aronsson J et al (2011) Dynamics of antibiotic resistant *Mycobacterium tuberculosis* during long-term infection and antibiotic treatment. *PLoS ONE* 6:e21147
- Martino MC, Rossi G, Martini I et al (2005) Mucosal lymphoid infiltrate dominates colonic pathological changes in murine experimental shigellosis. *J Infect Dis* 192:136–148
- Matsunaga T, Tomoda R, Nakajima T et al (1985) Photoelectrochemical sterilization of microbial cells by semiconductor powders. *FEMS Microbiol Lett* 29:211–214
- Matsunaga T, Tomoda R, Nakajima T et al (1988) Continuous-sterilization system that uses photoconductor powders. *Appl Environ Microbiol* 54:1330–1333
- Matusunga T (1985) Sterilization with particulate photoconductor. *J Antibact Antifung Agents* 13:211–220
- McDonnell G, Russell AD (1999) Antiseptics and disinfectants: activity, action, and resistance. *Clin Microbiol Rev* 12:147–179
- Nagaveni K, Hegde MS, Madras G (2004) Structure and photocatalytic activity of $\text{Ti}_{1-x}\text{M}_x\text{O}_{2\pm\delta}$ ($M = \text{W}, \text{V}, \text{Ce}, \text{Zr}, \text{Fe}, \text{and Cu}$) synthesized by solution combustion method. *J Phys Chem B* 108:20204–20212
- Navon-Venezia S, Ben-Ami R, Carmeli Y (2005) Update on *Pseudomonas aeruginosa* and *Acinetobacter baumannii* infections in the healthcare setting. *Curr Opin Infect Dis* 18:306–313
- Nazeeruddin MK, Humphry-Baker R, Liska P et al (2003) Investigation of sensitizer adsorption and the influence of protons on

- current and voltage of a dye-sensitized nanocrystalline TiO₂ solar cell. *J Phys Chem B* 107:8981–8987
- O'regan B, Gratzel M (1991) A low-cost, high-efficiency solar cell based on dye-sensitized colloidal TiO₂ films. *Nature* 353:737–740
- Pan X, Medina-Ramirez I, Mernaugh R et al (2010) Nanocharacterization and bactericidal performance of silver modified titania photocatalyst. *Colloids Surf B Biointerfaces* 77:82–89
- Ranjit KT, Willner I, Bossmann SH et al (2001) Lanthanide oxide-doped titanium dioxide photocatalysts: novel photocatalysts for the enhanced degradation of p-chlorophenoxyacetic acid. *Environ Sci Technol* 35:1544–1549
- Rehman S, Ullah R, Butt AM et al (2009) Strategies of making TiO₂ and ZnO visible light active. *J Hazard Mater* 170:560–569
- Russell AD (2003) Biocide use and antibiotic resistance: the relevance of laboratory findings to clinical and environmental situations. *Lancet Infect Dis* 3:794–803
- Salyers AA, Whitt DD (1994) In bacterial pathogenesis: a molecular approach. ASM Press, Washington, DC
- Setlow P (2005) Spores of *Bacillus subtilis*: their resistance to and killing by radiation, heat and chemicals. *J Appl Microbiol* 101:514–525
- Tsai TM, Chang HH, Chang KC et al (2010) A comparative study of the bactericidal effect of photocatalytic oxidation by TiO₂ on antibiotic-resistant and antibiotic-sensitive bacteria. *J Chem Technol Biotechnol* 85:1642–1653
- Tseng YH, Kuo CS, Huang CH et al (2006) Visible-light-responsive nano-TiO₂ with mixed crystal lattice and its photocatalytic activity. *Nanotechnology* 17:2490–2497
- Vohra A, Goswami DY, Deshpande DA et al (2005) Enhanced photocatalytic inactivation of bacterial spores on surfaces in air. *J Ind Microbiol Biotechnol* 32:364–370
- Wolfrum EJ, Huang J, Blake DM et al (2002) Photocatalytic oxidation of bacteria, bacterial and fungal spores, and model biofilm components to carbon dioxide on titanium dioxide-coated surfaces. *Environ Sci Technol* 36:3412–3419
- Wong HC, Liu SH, Wang TK et al (2000) Characteristics of *Vibrio parahaemolyticus* O3:K6 from Asia. *Appl Environ Microbiol* 66:3981–3986
- Wong MS, Chu WC, Sun DS et al (2006) Visible-light-induced bactericidal activity of a nitrogen-doped titanium photocatalyst against human pathogens. *Appl Environ Microbiol* 72:6111–6116
- Wong MS, Sun DS, Chang HH (2010) Bactericidal performance of visible-light responsive titania photocatalyst with silver nanostructures. *PLoS One* 5:e10394
- Wu P, Xie R, Imlay K et al (2010a) Visible-light-induced bactericidal activity of titanium dioxide codoped with nitrogen and silver. *Environ Sci Technol* 44:6992–6997
- Wu TS, Wang KX, Li GD et al (2010b) Montmorillonite-supported Ag/TiO₂ nanoparticles: an efficient visible-light bacteria photodegradation material. *ACS Appl Mater Interfaces* 2:544–550
- Yang MC, Yang TS, Wong MS (2004a) Nitrogen-doped titanium oxide films as visible light photocatalyst by vapor deposition. *Thin Solid Films* 469–470:1–5
- Yang TS, Shiu CB, Wong MS (2004b) Structure and hydrophilicity of titanium oxide films prepared by electron beam evaporation. *Surface Sci* 548:75–82
- Yang D, Fan T, Zhou H et al (2011) Biogenic hierarchical TiO/SiO₂ derived from rice husk and enhanced photocatalytic properties for dye degradation. *PLoS One* 6:e24788
- Yao Y, Ohko Y, Sekiguchi Y et al (2008) Self-sterilization using silicone catheters coated with Ag and TiO₂ nanocomposite thin film. *J Biomed Mater Res B Appl Biomater* 85:453–460
- Yu JC, Ho W, Yu J et al (2005) Efficient visible-light-induced photocatalytic disinfection on sulfur-doped nanocrystalline titania. *Environ Sci Technol* 39:1175–1179
- Zhang Z, Wang CC, Zakaria R et al (1998) Role of particle size in nanocrystalline TiO₂-based photocatalysts. *J Phys Chem B* 102:10871–10878
- Zhao W, Ma W, Chen C et al (2004) Efficient degradation of toxic organic pollutants with Ni₂O₃/TiO_{2-x}Bx under visible irradiation. *J Am Chem Soc* 126:4782–4783
- Zhu J, Zheng W, He B et al (2004) Characterization of Fe–TiO₂ photocatalysts synthesized by hydrothermal method and their photocatalytic reactivity for photodegradation of XRG dye diluted in water. *J Mol Catal A* 216:35–43
- Zhu J, Deng Z, Chen F et al (2006) Hydrothermal doping method for preparation of Cr³⁺-TiO₂ photocatalysts with concentration gradient distribution of Cr³⁺. *Appl Catal B* 62:329–335