

# Photocatalytic disinfection using titanium dioxide: spectrum and mechanism of antimicrobial activity

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**Abstract** The photocatalytic properties of titanium dioxide are well known and have many applications including the removal of organic contaminants and production of self-cleaning glass. There is an increasing interest in the application of the photocatalytic properties of TiO<sub>2</sub> for disinfection of surfaces, air and water. Reviews of the applications of photocatalysis in disinfection (Gamage and Zhang 2010; Chong et al., *Wat Res* 44 (10):2997–3027, 2010) and of modelling of TiO<sub>2</sub> action have recently been published (Dalrymple et al., *Appl Catal B* 98(1–2):27–38, 2010). In this review, we give an overview of the effects of photoactivated TiO<sub>2</sub> on microorganisms. The activity has been shown to be capable of killing a wide range of Gram-negative and Gram-positive bacteria, filamentous and unicellular fungi, algae, protozoa, mammalian viruses and bacteriophage. Resting stages, particularly bacterial endospores, fungal spores and protozoan cysts, are generally more resistant than the vegetative forms, possibly due to the increased cell wall thickness. The killing mechanism involves degradation of the cell wall and cytoplasmic membrane due to the production of reactive oxygen species such as hydroxyl radicals and hydrogen peroxide. This initially leads to leakage of cellular contents then cell lysis and may be followed by complete mineralisation of the organism. Killing is most efficient when there is close contact between the organisms and the TiO<sub>2</sub> catalyst. The killing

activity is enhanced by the presence of other antimicrobial agents such as Cu and Ag.

**Keywords** Antimicrobial · Disinfection · Mechanism · Photocatalysis · ROS · TiO<sub>2</sub> · Titania

## Introduction

The ability of titanium dioxide (titania, TiO<sub>2</sub>) to act as a photocatalyst has been known for 90 years (Renz 1921), and its role in the “chalking” of paint (formation of powder on the surface) is well known (Jacobsen 1949). Interest in the application of the photocatalytic properties of TiO<sub>2</sub> was revived when the photoelectrolysis of water was reported by Fujishima and Honda (1972), and this activity was soon exploited both for the ability to catalyse the oxidation of pollutants (Carey et al. 1976; Frank and Bard 1977) and the ability to kill microorganisms (Matusunga 1985; Matsunaga et al. 1985). Photocatalytic surfaces can be superhydrophilic, which means that water spreads on the surface, allowing dirt to be washed off, and commercial uses include self-cleaning windows (e.g. San Gobain Bioclean™, Pilkington Active™ and SunClean™; Chen and Poon 2009) and self-cleaning glass covers for highway tunnel lamps (Honda et al. 1998). There are currently over 11,000 publications on photocatalysis. Although an early study showed no improved antimicrobial activity of TiO<sub>2</sub> for disinfection of primary wastewater effluent (Carey and Oliver 1980), many subsequent studies have shown the usefulness of photocatalysis on TiO<sub>2</sub> for disinfection of water (Chong et al. 2010). These include killing of bacteria (Rincón and Pulgarin 2004a) and viruses from water supplies (Sjogren and Sierka 1994),

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tertiary treatment of wastewater (Araña et al. 2002), purifying drinking water (Wei et al. 1994; Makowski and Wardas 2001), treatment of wash waters from vegetable preparation (Selma et al. 2008) and in bioreactor design to prevent biofilm formation (Shiraishi et al. 1999). TiO<sub>2</sub>-coated filters have been used for the disinfection of air (Jacoby et al. 1998; Goswami et al. 1997, 1999; Lin and Li 2003a, b; Chan et al. 2005). The advantage of using photocatalysis along with conventional air filtration is that the filters are also self-cleaning. TiO<sub>2</sub> has also been used on a variety of other materials and applications (Table 1). The potential for killing cancer cells has also been evaluated (reviewed by Blake et al. 1999; Fujishima et al. 2000).

In recent years, there has been an almost exponential increase in the number of publications referring to photocatalytic disinfection (PCD), and the total number of publications now exceeds 800 (Fig. 1). Some of the early work was reviewed by Blake et al. (1999) and sections on photocatalytic disinfection have been included in several reviews (Mills and Le Hunte 1997; Fujishima et al. 2000, 2008; Carp et al. 2004); reviews of the use in disinfection of water (McCullagh et al. 2007; Chong et al. 2010) and modelling of TiO<sub>2</sub> action have been published (Dalrymple et al. 2010). In this

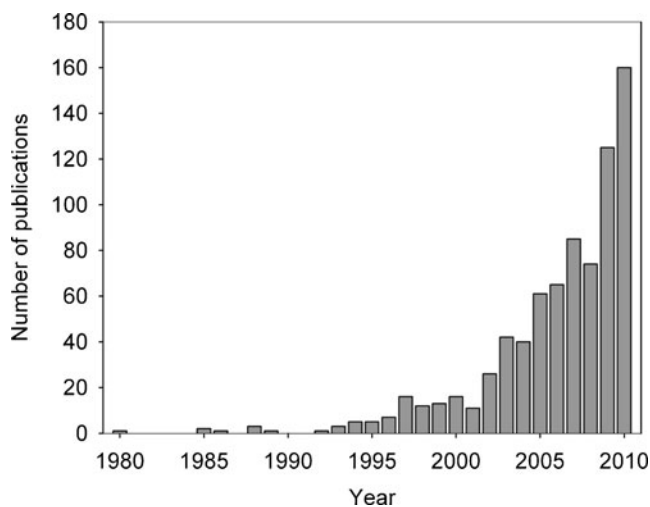
review, we explore the effects of photoactivated TiO<sub>2</sub> on microorganisms.

### Photocatalytic mechanism

For a more detailed discussion of the photochemistry, the reader is directed to the excellent reviews by Mills and Le Hunte (1997) and Hashimoto et al. (2005). TiO<sub>2</sub> is a semiconductor. The adsorption of a photon with sufficient energy by TiO<sub>2</sub> promotes electrons from the valence band ( $e_{vb}^-$ ) to the conduction band ( $e_{cb}^-$ ), leaving a positively charged hole in the valence band ( $h_{vb}^+$ ; Eq. 1). The band gap energy (energy required to promote an electron) of anatase is approx. 3.2 eV, which effectively means that photocatalysis can be activated by photons with a wavelength of below approximately 385 nm (i.e. UVA). The electrons are then free to migrate within the conduction band. The holes may be filled by migration of an electron from an adjacent molecule, leaving that with a hole, and the process may be repeated. The electrons are then free to migrate within the conduction band and the holes may be filled by an electron from an adjacent molecule. This process can be repeated. Thus, holes are also mobile. Electrons and holes may recombine (bulk recombination) a

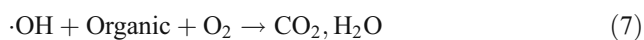
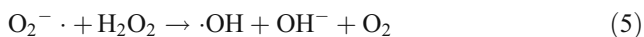
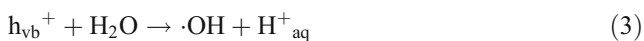
**Table 1** Some antimicrobial applications of TiO<sub>2</sub>

Uses and applications	Publication
Building materials, e.g. concrete	Guo et al. (2009) Chen and Poon (2009)
Catheters to prevent urinary tract infections	Ohko et al. (2001) Yao et al. (2008c)
Coatings for bioactive surfaces	Ueda et al. (2010)
Dental implants	Suketa et al. (2005) Mo et al. (2007)
Fabrics	Gupta et al. (2008), Kangwansupamonkon et al. (2009), Wu et al. (2009a, b), Yuranova et al. (2006)
Food packaging films	Chawengkijwanich and Hayata (2008)
Lancets	Nakamura et al. (2007)
Metal pins used for skeletal traction	Tsuang et al. (2008)
Orthodontic wires	Chun et al. (2007)
Paint	Allen et al. (2008)
Photocatalytic tiles for operating theatres	Fujishima et al. (1997)
Plastics	Paschoalino and Jardim (2008) Cerrada et al. (2008) Fujishima et al. (1997)
Protection of marble from microbial corrosion	Poulios et al. (1999)
Surgical face masks	Li et al. (2006)
Tent materials	Nimitrakoolchai and Supothina (2008)
TiO <sub>2</sub> -coated wood	Chen et al. (2009)
TiO <sub>2</sub> -containing paper	Geng et al. (2008)



**Fig. 1** Number of publications on photocatalytic disinfection

non-productive reaction, or, when they reach the surface, react to give reactive oxygen species (ROS) such as  $O_2^{\cdot-}$  (2) and  $\cdot OH$  (3). These in solution can react to give  $H_2O_2$  (4), further hydroxyl (5) and hydroperoxyl (6) radicals. Reaction of the radicals with organic compounds results in mineralisation (7). Bulk recombination reduces the efficiency of the process, and indeed some workers have applied an electric field to enhance charge separation, properly termed photoelectrocatalysis (Harper et al. 2000).



There are three main polymorphs of  $TiO_2$ : anatase, rutile and brookite. The majority of studies show that anatase was the most effective photocatalyst and that rutile was less active; the differences are probably due to differences in the extent of recombination of electron and hole between the two forms (Miyagi et al. 2004). However, studies have shown that mixtures of anatase and rutile were more

effective photocatalysts than 100% anatase (Miyagi et al. 2004) and were more efficient for killing coliphage MS2 (Sato and Taya 2006a). One active commercially available preparations of  $TiO_2$  is Degussa P25 (Degussa Ltd., Germany) which contains approx. 80% anatase and 20% rutile. The increased activity is generally ascribed to interactions between the two forms, reducing bulk recombination. Brookite has been relatively little studied, but a recent paper showed that a brookite–anatase mixture was more active than anatase alone (Shah et al. 2008). A silver-doped multiphase catalyst was shown to have increased photocatalytic activity, but its antimicrobial activity was not reported (Yu et al. 2005a). Indoor use of photocatalytic disinfection is limited by the requirement for UVA irradiation. Modified catalysts can reduce the band gap so that visible light activates the photocatalysis. This has been shown for  $TiO_2$  combined with C, N and S, metals such as Sn, Pd, and Cu, and dyes (Fujishima and Zhang 2006), but activity is generally lower than when activated with UVA. This area is currently the subject of much research.

The antimicrobial activity of UVA-activated  $TiO_2$  was first demonstrated by Matsunaga and coworkers (Matsunaga 1985; Matsunaga et al. 1985). Since then, there have been reports on the use of photocatalysis for the destruction of bacteria, fungi, algae, protozoa and viruses as well as microbial toxins.  $TiO_2$  can be used in suspension in liquids or immobilised on surfaces (Kikuchi et al. 1997; Sunada et al. 1998; Kühn et al. 2003; Yu et al. 2003a; Brook et al. 2007; Yates et al. 2008a, b; Ditta et al. 2008). The ability to eliminate microorganisms on photocatalytic self-cleaning/self-disinfecting surfaces may provide a useful additional mechanism in the control of transmission of diseases along with conventional disinfection methods. Copper and silver ions are well characterised for their antimicrobial activities and can also enhance the photocatalytic activity. Combinations of  $Cu^{2+}$  and  $Ag^+$  with  $TiO_2$  therefore provide dual function surfaces (see below).

### Photocatalytic action on microorganisms

Photocatalysis has been shown to be capable of killing a wide range of organisms including Gram-negative and Gram-positive bacteria, including endospores, fungi, algae, protozoa and viruses, and has also been shown to be capable of inactivating prions (Paspaltsis et al. 2006). Photocatalysis has also been shown to destroy microbial toxins. As far as the authors are aware, only *Acanthamoeba* cysts and *Trichoderma asperellum* conidiospores have been reported to be resistant (see below), but these have not been extensively studied. The ability to kill all other groups of microorganisms suggests that the surfaces have the potential to be self-sterilising, particularly when combined

with Cu or Ag. However, for the present, it is correct to refer to photocatalytic surfaces or suspensions as being self-disinfecting rather than self-sterilising. Many studies have used pure cultures, although there are reports of photocatalytic activity against mixed cultures (van Grieken et al. 2010) and of natural communities (Armon et al. 1998; Araña et al. 2002; Cho et al. 2007a).

#### Gram-negative bacteria

The great majority of studies have been performed with *Escherichia coli*, and there are far too many to give a complete list in this review. Some examples of different strains used and applications are shown in Table 2. Examples of other Gram-negative bacteria that are susceptible to PCD are shown in Table 3. They include cocci, straight and curved rods, and filamentous forms from 19 different genera.

#### Gram-positive bacteria

Most studies showed that Gram-positive bacteria were more resistant to photocatalytic disinfection than Gram-negative bacteria (Kim et al. 2003; Liu and Yang 2003; Erkan et al. 2006; Pal et al. 2005, 2007; Muszkat et al. 2005; Hu et al. 2007; Sheel et al. 2008; Skorb et al. 2008). The difference is usually ascribed to the difference in cell wall structure between Gram-positive and Gram-negative bacteria. Gram-negative bacteria have a triple-layer cell wall with an inner membrane (IM), a thin peptidoglycan layer (PG) and an outer membrane (OM), whereas Gram-positive bacteria have a thicker PG and no OM. However, a few studies show that Gram-positive bacteria were more sensitive. *Lactobacillus* was more sensitive than *E. coli* on a Pt-doped TiO<sub>2</sub> catalyst (Matsunaga et al. 1985). methicillin-resistant *Staphylococcus aureus* (MRSA) and *E. coli* were more resistant than *Micrococcus luteus* (Kangwansupamonkon et al. 2009). Dunlop et al. (2010) showed that MRSA were more sensitive than an extended spectrum  $\beta$ -lactamase (ESBL)-producing *E. coli* strain, but less sensitive than *E. coli* K12. *Enterococcus faecalis* was more resistant than *E. coli*, but more sensitive than *Pseudomonas aeruginosa* (Luo et al. 2008). Conversely, Kubacka et al. (2008a) showed no difference in sensitivity between clinical isolates of *P. aeruginosa* and *E. faecalis*. Van Grieken et al. (2010) saw no difference in disinfection time for *E. coli* and *E. faecalis* in natural waters, but *E. faecalis* was more resistant in distilled water. These differences may relate to different affinities for TiO<sub>2</sub> (close contact between the cells and the TiO<sub>2</sub> is required for optimal activity—see below) as well as cell wall structure.

Gram-positive bacteria that have been shown to be killed by PCD are shown in Table 4 and include species of 17

different genera, including aerobic and anaerobic endospore formers. The endospores were uniformly more resistant than the vegetative cells to PCD.

#### Fungi, algae and protozoa

Fungi, algae and protozoa that have been shown to be susceptible to PCD are shown in Tables 5 and 6. These include 11 genera of filamentous fungi, 3 yeasts, 2 amoebae, 1 Apicomplexan, 1 diplomonad, 1 ciliate and 7 algae, including 1 diatom. Fungal spores were generally more resistant than vegetative forms, and *Trichoderma harzianum* spores in particular were resistant to killing under the conditions tested (Giannantonio et al. 2009). Cysts of *Acanthamoeba* showed only a 50% reduction during the treatment time and may have been killed if the treatment time had been extended (Sökmen et al. 2008).

#### Viruses

Viruses that have been shown to be killed by PCD are shown in Table 7.

Most studies were on *E. coli* bacteriophages in suspension, which have been demonstrated for icosahedral ssRNA viruses (MS2 and Q $\beta$ ), filamentous ssRNA virus (fr), ssDNA (phi-X174) and dsDNA viruses ( $\lambda$  and T4). Other bacteriophages include *Salmonella typhimurium* phage PRD-1, *Lactobacillus* phage PL1 and an unspecified *Bacteroides fragilis* phage. Mammalian viruses include poliovirus 1, avian and human influenza viruses, and SARS coronavirus (Table 7).

#### Bacterial toxins

Photocatalytic activity has been shown to be capable of inactivating bacterial toxins including Gram-negative endotoxin and algal and cyanobacterial toxins (Table 8).

#### Mechanism of killing of bacteria

The mode of action of photoactivated TiO<sub>2</sub> against bacteria has been studied with both Gram-positive and Gram-negative bacteria. The killing action was originally proposed to be via depletion of coenzyme A by dimerization and subsequent inhibition of respiration (Matsunaga et al. 1985, 1988). However, there is overwhelming evidence that the lethal action is due to membrane and cell wall damage. These studies include microscopy, detection of lipid peroxidation products, leakage of intercellular components, e.g. cations, RNA and protein, permeability to low-molecular-weight labels, e.g. *o*-nitrophenyl-galactoside (ONPG), and spectroscopic studies.

**Table 2** Examples of *E. coli* strains shown to be killed by photocatalytic disinfection on TiO<sub>2</sub>

Organism	Notes	Reference
<i>Escherichia coli</i>	WO <sub>3</sub> nanoparticle doped TiO <sub>2</sub>	Tatsuma et al. (2003)
<i>Escherichia coli</i>	Degussa P25 impregnated cloth filter	Vohra et al. (2006)
<i>Escherichia coli</i> ATCC 8739	Degussa P25 suspension	Cho et al. (2005)
<i>Escherichia coli</i> ATCC 11229	Degussa P25 coated plexiglass	Kühn et al. (2003)
<i>Escherichia coli</i> ATCC 13706	Degussa P25 immobilised on glass substrate	Rodriguez et al. (2007)
<i>Escherichia coli</i> ATCC 10536	Ag and CuO – TiO <sub>2</sub> hybrid catalysts	Brook et al. (2007), Ditta et al. (2008)
<i>Escherichia coli</i> ATCC 15153	Degussa P25 suspension	Ibáñez et al. (2003)
<i>Escherichia coli</i> ATCC 23505	Rfc sputter was used to deposit films of 120 nm thickness onto glass and steel substrates	Shieh et al. (2006)
<i>Escherichia coli</i> ATCC 23631	Degussa P25 applied to a plastic support	Sichel et al. (2007a)
<i>Escherichia coli</i> ATCC 25922	Aldrich TiO <sub>2</sub> 99.9% pure anatase	Sökmen et al. (2001)
<i>Escherichia coli</i> ATCC 25922	Aerosol deposited nanocrystalline film	Ryu et al. (2008)
<i>Escherichia coli</i> ATCC 27325	Degussa P25, suspension	Huang et al. (2000) Maness et al. (1999)
<i>Escherichia coli</i> ATCC-39713	Aerosil P25 suspension	Matsunaga et al. (1995)
<i>Escherichia coli</i> CAH57 (ESBL)	Thin film TiO <sub>2</sub>	Dunlop et al. (2010)
<i>Escherichia coli</i> CCRC 10675	TiO <sub>2</sub> and ZnO suspension	Liu and Yang (2003)
<i>Escherichia coli</i> CECT 101	Sol–gel microemulsion with an Ag overlayer	Kubacka et al. (2008b)
<i>Escherichia coli</i> DH 4α	Degussa P25 suspension	Lan et al. (2007)
<i>Escherichia coli</i> DH5α	Flow through reactor Anatase thin film on glass	Belháčová et al. (1999) Yu et al. (2002, 2003b)
<i>Escherichia coli</i> HB101	Degussa P25 suspension	Bekbölet and Araz (1996), Bekbölet (1997)
<i>Escherichia coli</i> HB101	Degussa P25 and Ag/P25 mixed suspension	Coleman et al. (2005)
<i>Escherichia coli</i> IFO 3301	Silica coated lime glass plates dip coated with TiO <sub>2</sub>	Kikuchi et al. (1997) Sunada et al. (2003b)
<i>Escherichia coli</i> IM303	TiO <sub>2</sub> coated air filter	Sato et al. (2003)
<i>Escherichia coli</i> JM109	Anatase thin film on glass	Yu et al. (2002)
<i>Escherichia coli</i> K12 ATCC10798	Degussa P25 suspension	Duffy et al. (2004) McLoughlin et al. (2004a, b) Pal et al. (2007)
<i>Escherichia coli</i> K12 ATCC10798	Degussa P25 coated glass fibre air filter	Pal et al. (2008)
<i>Escherichia coli</i> K12 (ATCC 23716)	Degussa P25	Rincon and Pulgarin (2003, 2004a)
<i>Escherichia coli</i> K12 (ATCC 2363)	Degussa P25 suspension	Marugan et al. (2008)
<i>Escherichia coli</i> K12	Degussa P25 suspension	Fernandez et al. (2005) Gumy et al. (2006a, b) Quisenberry et al. (2009)
<i>Escherichia coli</i> K12	Thin film TiO <sub>2</sub>	Dunlop et al. (2002)
<i>Escherichia coli</i> MG1655	Degussa P25 suspension	Gogniat and Dukan (2007)
<i>Escherichia coli</i> MM294	Degussa P25 suspension	Kim et al. (2004)
<i>Escherichia coli</i> NCIMB-4481	Immobilised TiO <sub>2</sub>	Butterfield et al. (1997)
<i>Escherichia coli</i> PHL1273	Degussa P25 suspension	Benabbou et al. (2007)
<i>Escherichia coli</i> PHL1273	Degussa P25 and millennium PC500	Guillard et al. (2008)
<i>Escherichia coli</i> S1400/95	Degussa P25 suspension	Robertson et al. (2005)
<i>Escherichia coli</i> 078	Thin films on glass substrate	Choi et al. (2004)
<i>Escherichia coli</i> XL1 Blue MRF	Anatase thin film on glass	Yu et al. (2002)

**Table 3** Other Gram-negative bacteria shown to be killed by photocatalytic disinfection

Organism	Notes	Reference
<i>Acinetobacter</i>	TiO <sub>2</sub> suspension	Kashyout et al. (2006)
<i>Acinetobacter baumannii</i>	C doped TiO <sub>2</sub>	Cheng et al. (2009)
<i>Aeromonas hydrophila</i> AWWX1	TiO <sub>2</sub> pellets	Kerstens et al. (1998)
<i>Anabaena</i>	TiO <sub>2</sub> -coated glass beads	Kim and Lee (2005)
<i>Bacteroides fragilis</i>	TiO <sub>2</sub> on orthopaedic implants	Tsuang et al. (2008)
<i>Coliforms</i>	Degussa P25 suspension	Araña et al. (2002)
<i>Coliforms</i>	Anatase suspension	Watts et al. (1995)
<i>Edwardsiella tarda</i>	Sol/gel-coated glass slides	Cheng et al. (2008)
<i>Enterobacter aerogenes</i>	Degussa P25 suspension	Ibáñez et al. (2003)
<i>Enterobacter cloacae</i> SM1	Anatase, spin-coated glass plates	Yao et al. (2007a)
<i>Erwinia carotovora</i> subsp. <i>carotovora</i>	Degussa P25 suspension	Muszkat et al. (2005)
<i>Erwinia carotovora</i> subsp. <i>carotovora</i> ZL1, subsp. <i>Carotovora</i> 3, subsp. <i>Carotovora</i> 7	Anatase, spin-coated glass lates	Yao et al. (2007a, b, 2008a, b)
Faecal coliforms	Anatase suspension	Watts et al. (1995)
<i>Flavobacterium</i> sp.	TiO <sub>2</sub> suspension and coated glass beads	Cohen-Yaniv et al. (2008)
<i>Fusobacterium nucleatum</i>	Thin film of anatase on titanium	Suketa et al. (2005), Bai et al. (2007)
<i>Legionella pneumophila</i> ATCC 33153	Degussa P25 suspension	Cheng et al. (2007)
<i>Legionella pneumophila</i> CCRC 16084	TiO <sub>2</sub> air filter + UVC	Li et al. (2003)
<i>Legionella pneumophila</i> GIFU-9888	Ultrasonic activated suspension of TiO <sub>2</sub>	Dadjour et al. (2005, 2006)
<i>Microcystis</i>	TiO <sub>2</sub> -coated glass beads	Kim and Lee (2005)
<i>Porphyromonas gingivalis</i>	TiO <sub>2</sub> sol/gel-coated orthodontic wires	Chun et al. (2007)
<i>Prevotella intermedia</i>	Ag-hydroxyapatite-TiO <sub>2</sub> catalyst	Mo et al. (2007)
<i>Proteus vulgaris</i>	P25 (10% Pt), 0.25 g/L slurry	Matsunaga et al. (1985)
<i>P. aeruginosa</i>	Surfaces	Kühn et al. (2003)
<i>P. aeruginosa</i> environmental isolate	Spray-coated soda lime glass and silica tubing	Amezaga-Madrid et al. (2002, 2003)
<i>P. aeruginosa</i> PA01	Thin film	Gage et al. (2005)
<i>P. aeruginosa</i>	Coated Al fibres	Luo et al. (2008)
<i>P. aeruginosa</i>	Catheters	Yao et al. (2008c)
<i>P. fluorescens</i> R2F	TiO <sub>2</sub> pellets	Kerstens et al. (1998)
<i>P. fluorescens</i> B22	Sigma-Aldrich TiO <sub>2</sub> thin films	Skorb et al. (2008)
<i>Pseudomonas</i> sp.	Anodized titanium alloy	Muraleedharan et al. (2003)
<i>Pseudomonas stutzeri</i> NCIMB11358	TiO <sub>2</sub> suspension	Biguzzi and Shama (1994)
<i>Pseudomonas syringae</i> pv tomato	Degussa P25 suspension	Muszkat et al. (2005)
<i>Pseudomonas tolaasi</i>	TiO <sub>2</sub> suspension	Sawada et al. (2005)
<i>Salmonella choleraesuis</i>	Anatase suspension	Kim et al. (2003)
<i>Salmonella enteritidis</i> Typhimurium	Degussa P25 suspension	Ibáñez et al. (2003), Cushnie et al. (2009)
<i>Salmonella enteritidis</i> Typhimurium	TiO <sub>2</sub> film on quartz rods with UVC	Cho et al. (2007a, b)
<i>Serratia marcescens</i>	Degussa P25 suspension	Block et al. (1997)
		Goswami et al. (1999)
<i>Shigella flexneri</i>	C-doped TiO <sub>2</sub>	Cheng et al. (2009)
<i>Vibrio parahaemolyticus</i>	Anatase suspension	Kim et al. (2003)
<i>Vibrio parahaemolyticus</i> VP 144	Anatase TiO <sub>2</sub> dip coated on open porcelain filter cell	Hara-Kudo et al. (2006)
<i>Vibrio vulnificus</i>	TiO <sub>2</sub> -impregnated steel fibres for water treatment	Song et al. (2008)

### Changes in cell permeability

Indirect evidence for membrane damage comes from studies of leakage of cellular components. Saito et al.

(1992) showed that there was a rapid leakage of K<sup>+</sup> from treated cells of *Streptococcus sobrinus* AHT which occurred within 1 min of exposure and paralleled the loss of viability. This was followed by a slower release of RNA

**Table 4** Gram-positive bacteria shown to be killed by photocatalytic disinfection

Organism	Notes	Reference
<i>Actinobacillus actinomycetemcomitans</i>	TiO <sub>2</sub> coating on titanium	Suketa et al. (2005)
<i>Actinomyces viscosus</i>	Kobe Steel TiO <sub>2</sub> 99.98% anatase	Nagame et al. (1989)
<i>Bacillus cereus</i>	TiO <sub>2</sub> suspension	Cho et al. (2007a)
<i>Bacillus cereus</i> spores	TiO <sub>2</sub> suspension	Armon et al. (2004)
<i>Bacillus megaterium</i> QM B1551	Colloidal suspension of TiO <sub>2</sub>	Fu et al. (2005)
<i>Bacillus pumilis</i> spores ATCC 27142	TiO <sub>2</sub> anatase 99.9% slurry in Petri dish	Pham et al. (1995, 1997)
<i>Bacillus</i> sp.	Degussa P-25 immobilised on Pyrex glass	Rincón and Pulgarin (2005)
<i>Bacillus subtilis</i> vegetative cells and endospores	Degussa P25-coated quartz discs	Wolfrum et al. (2002)
<i>Bacillus subtilis</i> endospores	Aluminium foil coated with TiO <sub>2</sub>	Greist et al. (2002)
<i>Bacillus thuringiensis</i>	100% anatase thin film ± Pt doping	Kozlova et al. (2010)
<i>Clavibacter michiganensis</i>	Solar + H <sub>2</sub> O <sub>2</sub>	Muszkat et al. (2005)
<i>Clostridium difficile</i>	Evonik Aeroxide P25 thin film	Dunlop et al. (2010)
<i>Clostridium perfringens</i> spores NCIMB 6125	TiO <sub>2</sub> film on metal electrode	Butterfield et al. (1997)
<i>Clostridium perfringens</i> spores	Degussa P-25 + UVC	Guimarães and Barretto (2003)
<i>Deinococcus radiophilus</i>	TiO <sub>2</sub> suspension	Laot et al. (1999)
<i>Enterococcus (Streptococcus) faecalis</i>	Degussa P25 suspension	Herrera Melián et al. (2000)
<i>Enterococcus (Streptococcus) faecalis</i>	Immobilised TiO <sub>2</sub>	Singh et al. (2005)
<i>Enterococcus faecalis</i> CECT 481	Degussa P25 suspension	Vidal et al. (1999)
<i>Enterococcus faecium</i>	Degussa P25-coated Plexiglass	Kühn et al. (2003)
<i>Enterococcus hirae</i>	TiO <sub>2</sub> on orthopaedic implants	Tsuang et al. (2008)
<i>Enterococcus</i> sp.	Degussa P-25 suspension	Rincón and Pulgarin (2005)
<i>Lactobacillus acidophilus</i>	Degussa P25 suspension	Matsunaga et al. (1985), Choi et al. (2007a)
<i>Lactobacillus helveticus</i> CCRC 13936	TiO <sub>2</sub> suspension	Liu and Yang (2003)
<i>Lactococcus lactis</i> 411	Sigma-Aldrich TiO <sub>2</sub> thin films	Skorb et al. (2008)
<i>Listeria monocytogenes</i>	TiO <sub>2</sub> (Yakuri Pure Chemical Company, Japan) suspension	Kim et al. (2003)
<i>Microbacterium</i> sp. Microbacteriaceae str. W7	Degussa P25 immobilised on membrane	Pal et al. (2007)
<i>Micrococcus luteus</i>	Degussa P25 thick film	Wolfrum et al. (2002)
<i>Micrococcus lylae</i>	TiO <sub>2</sub> suspension	Yu et al. (2005b)
MRSA	Fe <sub>3</sub> O <sub>4</sub> -TiO <sub>2</sub> core/shell magnetic nanoparticles in suspension	Chen et al. (2008)
MRSA	TiO <sub>2</sub> thin film on titanium	Oka et al. (2008)
<i>Mycobacterium smegmatis</i>	100% anatase thin film ± Pt doping	Kozlova et al. (2010)
<i>Porphyromonas gingivalis</i>	TiO <sub>2</sub> thin film on steel and titanium	Shiraishi et al. (1999)
<i>Paenibacillus</i> sp SAFN-007	Degussa P25 immobilised on membrane	Pal et al. (2007)
<i>Staphylococcus aureus</i>	Degussa P25 suspension	Block et al. (1997)
<i>Staphylococcus aureus</i>	TiO <sub>2</sub> thin film on steel and titanium	Shiraishi et al. (1999)
<i>Staphylococcus epidermidis</i> NCTC11047	Ag-TiO <sub>2</sub> catalyst	Sheel et al. (2008)
<i>Staphylococcus saprophyticus</i>	Fe <sub>3</sub> O <sub>4</sub> -TiO <sub>2</sub> core/shell magnetic nanoparticles in suspension	Chen et al. (2008)
<i>Streptococcus cricetus</i>	Kobe Steel TiO <sub>2</sub> 99.98% anatase	Nagame et al. (1989)
<i>Streptococcus iniae</i>	Sol/gel-coated glass slides	Cheng et al. (2008)
<i>Streptococcus mutans</i>	TiO <sub>2</sub> sol/gel-coated orthodontic wires	Chun et al. (2007)
<i>Streptococcus mutans</i> GS5, LM7, OMZ175	P25 aerosil, 70% anatase suspension	Saito et al. (1992)
<i>Streptococcus pyogenes ery<sup>r</sup> cam<sup>r</sup></i>	Fe <sub>3</sub> O <sub>4</sub> -TiO <sub>2</sub> core/shell magnetic nanoparticles in suspension	Chen et al. (2008)
<i>Streptococcus rattus</i> FA-1	P25 aerosil, 70% anatase suspension	Saito et al. (1992)
<i>Streptococcus sobrinus</i> AHT	P25 suspension	Saito et al. (1992)

**Table 5** Fungi shown to be killed by photocatalytic disinfection

Organism	Notes	Reference
<i>Aspergillus niger</i> AS3315	Wood coated with TiO <sub>2</sub>	Chen et al. (2009)
<i>A. niger</i> spores	Degussa P25 film on quartz discs	Wolfrum et al. (2002)
<i>Aspergillus niger</i>	Thin films of TiO <sub>2</sub> on glass plates	Erkan et al. (2006)
<i>Candida albicans</i> ATCC 10231	Degussa P25 suspension	Lonnen et al. (2005)
<i>Candida albicans</i>	TiO <sub>2</sub> -coated surfaces	Kühn et al. (2003)
<i>Candida famata</i>	TiO <sub>2</sub> coated catheters	Yao et al. (2008c)
<i>Candida vini</i>	TiO <sub>2</sub> thin film	Veselá et al. (2008)
<i>Cladobotryum varium</i>	TiO <sub>2</sub> suspension	Sawada et al. (2005)
<i>Cladosporium cladosporioides</i>	TiO <sub>2</sub> -coated concrete	Giannantonio et al. (2009)
<i>Diaporthe actinidae</i>	TiO <sub>2</sub> immobilised on alumina spheres	Hur et al. (2005)
<i>Erysiphe cichoracearum</i>	Degussa P25 and Ce <sup>3+</sup> doped catalyts	Lu et al. (2006)
<i>Epicoccum nigrum</i>	TiO <sub>2</sub> coated concrete	Giannantonio et al. (2009)
Fungi from spinach	Plastic fruit containers with TiO <sub>2</sub> coating	Koide and Nonami (2007)
<i>Fusarium mucor</i>	TiO <sub>2</sub> -coated concrete	Giannantonio et al. (2009)
<i>Fusarium solani</i> ATCC 36031	Degussa P25 suspension	Lonnen et al. (2005)
<i>Fusarium</i> spp. ( <i>equisetii</i> , <i>oxyartan</i> , <i>anthophilum</i> , <i>verticilloides</i> , <i>solani</i> )	TiO <sub>2</sub> suspension, solar irradiation	Sichel et al. (2007b, c)
<i>Hanseula anomala</i> CCY-138-30	TiO <sub>2</sub> - and Ag-doped	Veselá et al. (2008)
<i>Peronophythora litchii</i>	Degussa P25- and Ce <sup>3+</sup> -doped catalyts	Lu et al. (2006)
<i>Penicillium citrinum</i>	TiO <sub>2</sub> -coated air filter	Lin and Li (2003a, b)
<i>Penicillium expansum</i>	TiO <sub>2</sub> spray coated on polypropylene film	Maneerat and Hayata (2006)
<i>Penicillium oxalicum</i>	TiO <sub>2</sub> -coated concrete	Giannantonio et al. (2009)
<i>Pestotiopsis maculans</i>	TiO <sub>2</sub> -coated concrete	Giannantonio et al. (2009)
<i>Saccharomyces cerevisiae</i>	Aerosil P25 suspension	Matsunaga et al. (1985)
<i>Sacchararomyces cerevisiae</i>	Pd-doped TiO <sub>2</sub>	Erkan et al. (2006)
<i>Spicellum roseum</i>	TiO <sub>2</sub> suspension	Sawada et al. (2005)
<i>Trichoderma asperellum</i>	TiO <sub>2</sub> -coated concrete	Giannantonio et al. (2009)
<i>Trichoderma harzianum</i>	TiO <sub>2</sub> suspension	Sawada et al. (2005)

and protein. Leakage of K<sup>+</sup> was also shown to parallel cell death of *E. coli* (Hu et al. 2007; Kambala and Naidu 2009). Huang et al. (2000) showed an initial increase in permeability to small molecules such as ONPG which was followed by leakage of large molecules such as  $\beta$ -D-galactosidase from treated cells of *E. coli*, suggesting a progressive increase in membrane permeability. Membrane damage has been shown with cells labelled with the LIVE-DEAD<sup>®</sup> BacLight<sup>™</sup> Bacterial Viability Kit which uses the fluorescent dyes Cyto 9, which stains all cells green, and propidium iodide, which only penetrates cells with damaged membranes and stains cells red. Gogniat et al. (2006) showed that permeability changes occurred in the membrane soon after attachment of *E. coli* to the TiO<sub>2</sub>, and we have seen similar changes (Ditta and Foster, unpublished). However, no damage was detected on a visible light active PdO/TiON catalyst until the catalyst had been irradiated (Wu et al. 2010b). SEM clearly showed membrane damage after irradiation on this catalyst (Wu et al. 2008, 2009a, b, 2010b; see Fig. 2).

#### Microscopic changes during PCD

TEM images of treated cells of *S. sobrinus* showed clearly that the cell wall was partially broken after cells had undergone TiO<sub>2</sub> photocatalytic treatment for 60 min, with further disruption after 120 min (Saito et al. 1992). The authors suggested that cell death was caused by alterations in cell permeability and the decomposition of the cell wall. SEM images of *S. aureus*, MRSA, *E. coli* and *M. luteus* showed morphological changes suggestive of cell wall disruption after UVA irradiation on apatite-coated TiO<sub>2</sub> on cotton fabrics (Kangwansupamonkon et al. 2009).

Damage to the cell wall of *P. aeruginosa* was shown by SEM and TEM, which showed changes in membrane structure such as “bubble-like protuberances which expelled cellular material” (Fig. 3; Amezaga-Madrid et al. 2002, 2003). They suggested that leakage of cellular material, and possibly abnormal cell division, was occurring, although the bubbles may have been due to localised



**Table 6** Protozoa and algae shown to be killed by photocatalytic disinfection

Organism	Notes	Reference
<b>Protozoa</b>		
<i>Acanthamoeba castellanii</i>	Degussa P25 suspension Only 50% kill for cysts, trophozoites were sensitive	Sökmen et al. (2008)
<i>Acanthamoeba polyphaga</i> environmental isolate	Degussa P25 suspension	Lonnen et al. (2005)
<i>Cryptosporidium parvum</i>	UVC + TiO <sub>2</sub>	Ryu et al. (2008)
<i>Cryptosporidium parvum</i>	Sol-gel and thermal TiO <sub>2</sub> thin films applied to Petri dish with a counter electrode Pt mesh	Curtis et al. (2002)
<i>Giardia</i> sp.	Fibrous ceramic TiO <sub>2</sub> filter	Navalon et al. (2009)
<i>Giardia intestinalis</i> cysts	TiO <sub>2</sub> (anatase 99.9%) + Ag <sup>+</sup>	Sökmen et al. (2008)
<i>Giardia lamblia</i>	TiO <sub>2</sub> thin film catalyst	Lee et al. (2004)
<i>Tetrahymena pyriformis</i>	TiO <sub>2</sub> suspension	Peng et al. (2010)
<b>Algae</b>		
<i>Amphidinium corterae</i>	Ag-TiO <sub>2</sub> catalyst	Rodriguez-Gonzalez et al. (2010)
<i>Chlorella vulgaris</i>	TiO <sub>2</sub> -Pt catalyst	Matsunaga et al. (1985)
<i>Cladophora</i> sp.	TiO <sub>2</sub> -covered glass beads	Peller et al. (2007)
<i>Chroococcus</i> sp. 27269	Anatase, fluorescent light	Hong et al. (2005)
<i>Melosira</i> sp.	TiO <sub>2</sub> -coated glass beads	Kim and Lee (2005)
<i>Oedogonium</i> sp.	TiO <sub>2</sub> -coated concrete	Linkous et al. (2000)
<i>Tetraselmis suecica</i>	Ag-TiO <sub>2</sub> catalyst	Rodriguez-Gonzalez et al. (2010)

damage to the peptidoglycan layer allowing the inner membrane to bulge through the peptidoglycan layer. Sunada et al. (2003b) studied killing of *E. coli* on thin films of TiO<sub>2</sub> and showed that the outer membrane was

damaged first and then the cytoplasmic membrane followed by complete degradation. Photocatalytic killing occurred without substantial visible degradation of peptidoglycan. Atomic force microscopy measurements of cells on

**Table 7** Viruses shown to be killed by photocatalytic disinfection

Host	Virus	Reference
<i>Bacteroides fragilis</i>	Not specified	Armon et al. (1998)
Birds	Influenza (avian) A/H5N2	Guillard et al. (2008)
<i>E. coli</i>	Coliphage	Guimarães and Barretto (2003)
<i>E. coli</i>	<i>φ</i> r	Gerrity et al. (2008)
<i>E. coli</i>	T4	Ditta et al. (2008), Sheel et al. (2008)
<i>E. coli</i>	λ vir	Yu et al. (2008)
<i>E. coli</i>	λNM1149	Belhácová et al. (1999)
<i>E. coli</i>	φX174	Gerrity et al. (2008)
<i>E. coli</i>	MS2	Sjogren and Sierka (1994), Greist et al. (2002), Cho et al. (2004, 2005), Sato and Taya (2006a, b), Vohra et al. (2006), Gerrity et al. (2008)
<i>E. coli</i>	Qβ	Lee et al. (1997), Otaki et al. (2000)
Human	Hepatitis B virus surface antigen HBsAg	Zan et al. (2007)
Human	Influenza A/H1N1	Lin et al. (2006)
Human	Influenza A/H3N2	Kozlova et al. (2010)
Human	Norovirus	Kato et al. (2005)
Human	<i>Poliovirus</i> type 1 (ATCC VFR-192)	Watts et al. (1995)
Human	SARS coronavirus	Han et al. (2004)
Human	Vaccinia	Kozlova et al. (2010)
<i>Lactobacillus casei</i>	PL-1	Kakita et al. (1997, 2000), Kashige et al. (2001)
<i>Salmonella typhimurium</i>	PRD1	Gerrity et al. (2008)

**Table 8** Microbial toxins inactivated by photocatalysis

Toxin	Publication
Brevetoxins	Khan et al. (2010)
Cylindrospermopsin	Senogles et al. (2000, 2001)
Lipopolysaccharide endotoxin	Sunada et al. (1998)
Microcystin-LR	Lawton et al. (1999, 2003) Cornish et al. (2000) Feitz and Waite (2003) Choi et al. (2007b)
Microcystins LR, YA and YR	Shephard et al. (1998)
Nodularin	Liu et al. (2005)

illuminated TiO<sub>2</sub> film showed that the outer membrane decomposed first (Sunada et al. 2003b).

TEM images showed progressive destruction of *E. coli* cells on Ag/AgBr/TiO<sub>2</sub> in suspension (Hu et al. 2006). Cell membrane was degraded first followed by penetration of TiO<sub>2</sub> particles into the cell and further damage. TEM of *E. coli* showed that there were changes to the nucleoid which became condensed, possibly due to leakage of ions out of the cell (Chung et al. 2009).

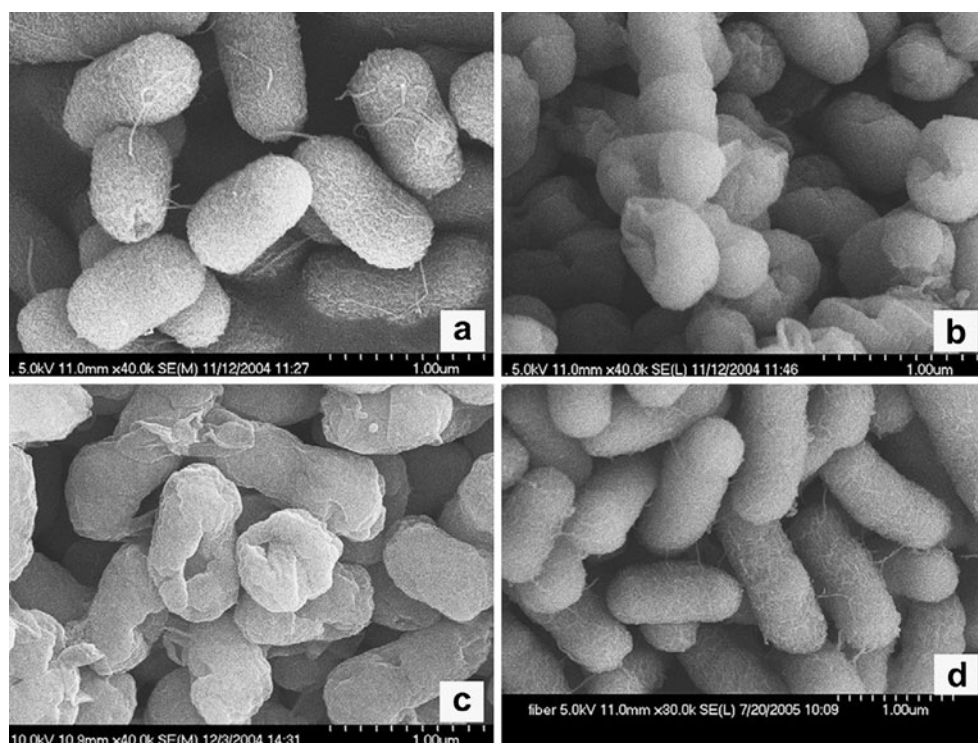
TEM of thin sections of treated cells of *E. coli* on a visible light-activated TiO<sub>2</sub> showed various degrees of cell disruption including plasmolysis, intracellular vacuoles ghost and cell debris (Vacaroiu et al. 2009). SEM and TEM studies showed initial swelling and rough appearance of the cells followed by scars and holes in the OM,

especially where the TiO<sub>2</sub> particles were in contact with the cells. Erdem et al. (2006) showed damage by SEM on *E. coli* and production of membrane breakdown products. SEM has shown changes to the outer membrane of *E. coli* (Li et al. 2008; Shah et al. 2008; Gartner et al. 2009). TEM of thin sections of treated cells of *E. coli* on a visible light-activated TiO<sub>2</sub> showed various degrees of cell disruption including plasmolysis, intracellular vacuoles ghost and cell debris (Vacaroiu et al. 2009).

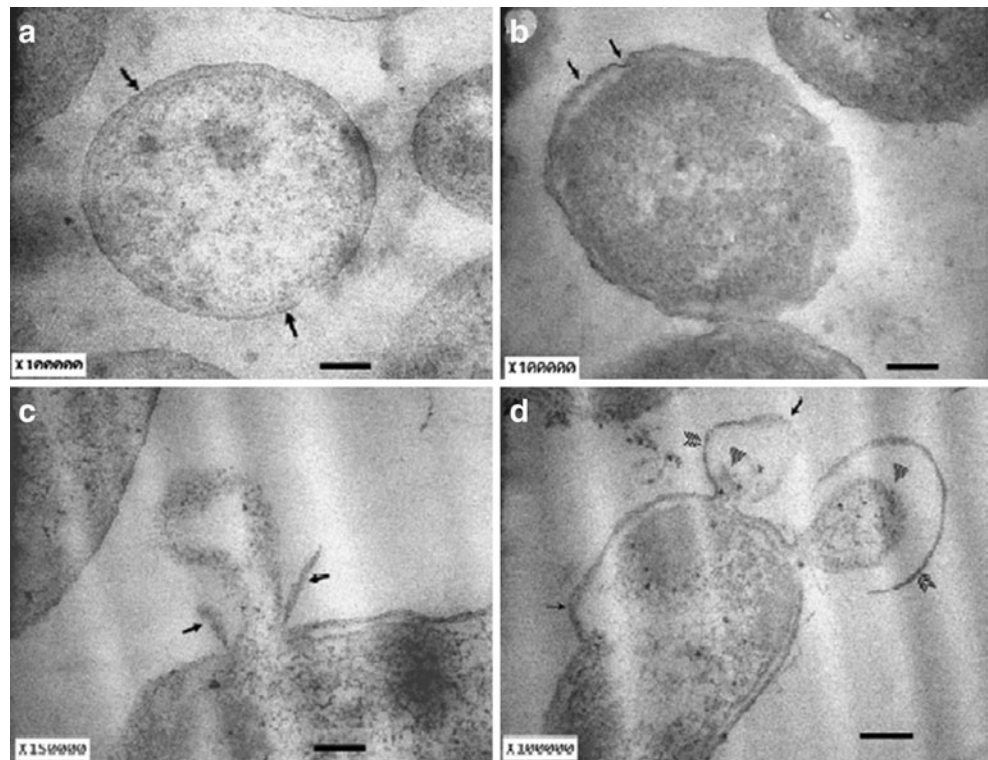
Atomic force microscopy was used to show membrane damage to *E. coli*, *S. aureus* and *Diplococcus (Streptococcus) pneumoniae* on thin films of TiO<sub>2</sub> (Miron et al. 2005). Changes to treated cells of *S. aureus* seen by TEM included separation of cytoplasmic membrane from the peptidoglycan layer (Chung et al. 2009). Distortion of treated cells of both MRSA and methicillin-sensitive *S. aureus* was seen by SEM on anatase–brookite (Shah et al. 2008), again suggesting cell wall damage.

Lipid peroxidation by ROS was demonstrated by the release of MDA as a breakdown product, and there was a concurrent loss of membrane respiratory activity measured by reduction of 2,3,5-triphenyltetrazolium chloride (Maness et al. 1999). The demonstration of degradation of *E. coli* endotoxin without substantial degradation of peptidoglycan (Sunada et al. 1998) suggested that in the case of Gram-negative bacteria, cell disruption occurred in the order of OM→PG→IM. However, alterations to the peptidoglycan layer may not be obvious in electron micrographs as peptidoglycan is a highly cross-linked structure and

**Fig. 2** Scanning electron micrographs of photocatalytically treated *E. coli*. **a** Untreated cells. **b, c** Cells after 240 min. **d** Cells after 30 min. Catalyst TiON thin film. From Wu et al. (2010a, b)



**Fig. 3** Transmission electron micrographs of photocatalytically treated *P. aeruginosa*. Untreated cells transverse section showing normal thickness and shape cell wall (arrows). **b–d** Cells after 240 min treatment showing abnormal wavy cell wall (arrows) (**b**), cytoplasmic material escaping from the cell with damaged cell wall (arrows) (**c**) and cell showing two “bubbles” of cellular material with cell wall (arrows) (**d**). Catalyst TiO<sub>2</sub> thin film. Bar marker=200 nm. From Amezaga-Madrid et al. (2003b)



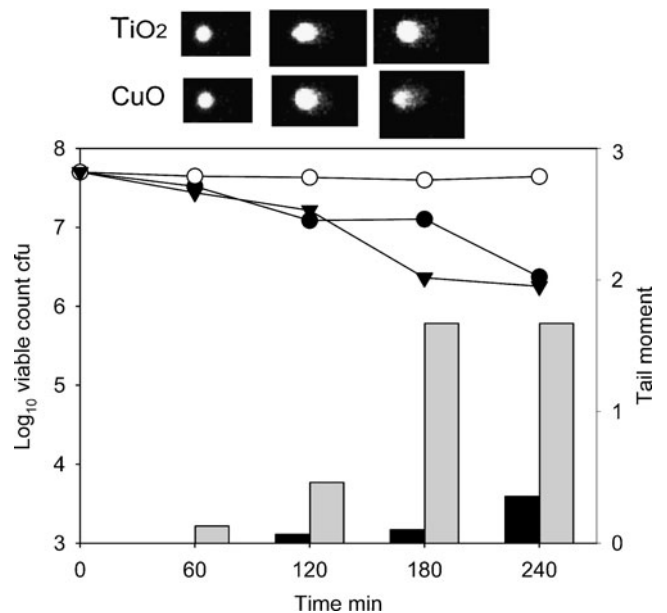
appreciable damage may occur without destruction of its overall appearance. Localised destruction may occur where TiO<sub>2</sub> particles are in contact with the cell. This may allow protrusion of inner membrane through the cell wall as seen by Amezaga-Madrid et al. (2003), followed by total rupture of the cell wall.

Yao et al. (2007c) showed damage to cells of *Erwinia carotovora* and DNA damage, which suggested that damage to DNA was responsible for cell death. However, our own data showed that there was no DNA damage seen by COMET assay on plain TiO<sub>2</sub> surfaces even when 97% of the cells were non-viable (Varghese and Foster, unpublished data; Fig. 4). Damage to DNA does occur on TiO<sub>2</sub> (Wamer et al. 1997; Hirakawa et al. 2004; Wang and Yang 2005; Wang et al. 2005; Gogniat and Dukan 2007; Shen et al. 2008; Yao et al. 2007c; Yang and Wang 2008), but is probably a late event after rupture of the membrane and cell death.

#### Killing of other microorganisms

There have been fewer studies on the mechanism of killing of eukaryotes. Linkous et al. (2000) suggested that death of the alga *Oedogonium* sp. was due to nonspecific breakdown of cellular structures. Microscopy has shown membrane damage to the alga *Chroococcus* sp. (Hong et al. 2005). Light microscopy and SEM showed damage to cell walls of *Candida albicans* suspended over a thin film of TiO<sub>2</sub> (Kühn et al. 2003) and on TiO<sub>2</sub>-coated tissue conditioner (Akiba et al. 2005). Cell wall and membrane

damage to cysts were seen with light microscopy of photocatalytically treated *Giardia lamblia* (Sökmen et al. 2008). Membrane damage was also shown to occur on



**Fig. 4** Comet assay of DNA from cells of *E. coli* on photoirradiated TiO<sub>2</sub> and CuO–TiO<sub>2</sub> catalysts. Upper photographs show fragmented DNA entering the gel like the tail of a comet. The graph shows viability (control, open circle; TiO<sub>2</sub> catalyst, closed circle; TiO<sub>2</sub>–CuO dual catalyst, downturned triangle) and tail moment (TM = Tail length × % DNA in tail/100; Olive et al. 1990) as the measure of the extent of DNA damage (TiO<sub>2</sub> catalyst, black square; TiO<sub>2</sub>–CuO dual catalyst, gray square) against time

treatment of the ciliate protozoan *Tetrahymena pyriformis* (Peng et al. 2010).

Killing of *Lactobacillus* phage PL1 by thin films of TiO<sub>2</sub> suspended in liquid was reported to be via initial damage to protein of the capsid by ·OH, followed by damage to the phage DNA inside the particles (Kashige et al. 2001). SEM showed ghost particles and empty heads. Damage to the H and N projections of influenza virus A/H1N1 occurred on PCD and was followed by total mineralisation (Lin et al. 2006).

#### Spectroscopic studies

The activity of titanium dioxide on isolated phospholipid bilayers has been shown to result in disruption of the bilayer structure using X-ray diffraction (Suwalsky et al. 2005), laser kinetic spectroscopy and attenuated total reflection Fourier transform infrared spectroscopy (FTIR). Disruption was shown to be due to lipid peroxidation (Kiwi and Nadochenko 2004; Nadochenko et al. 2006) measured by production of malondialdehyde (MDA). Lipid peroxidation occurs when polyunsaturated fatty acids such as linoleic acid are attacked by ROS (Kiwi and Nadochenko 2005).

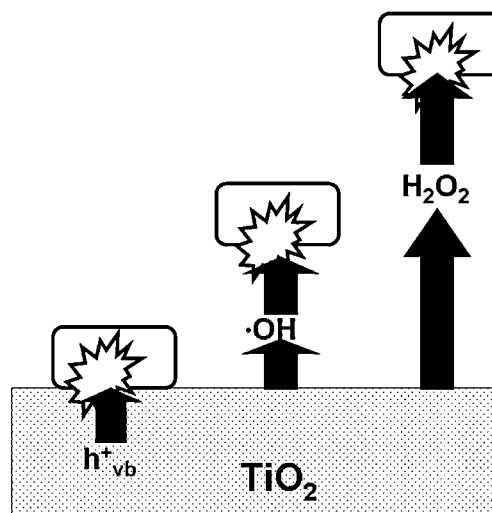
FTIR spectra of treated *E. coli* confirmed the production of carboxylic acids such as MDA as products of membrane degradation. MDA was further degraded by longer irradiation times (Hu et al. 2007).

The electron decay on TiO<sub>2</sub> was studied using laser kinetic spectroscopy in the presence of phosphatidyl ethanolamine, lipopolysaccharide and *E. coli* (Nadochenko et al. 2006). Spectroscopic studies using FTIR spectroscopy suggested that organic components bound to the TiO<sub>2</sub> were directly oxidised by reduction of the electron holes (Nadochenko et al. 2006, 2008). This work suggested that direct oxidation of cellular components could occur without the production of ROS, but only if cells were in direct contact with the surface of the TiO<sub>2</sub>. This is wholly consistent with the greater effectiveness of PCD when the cells are in contact with the TiO<sub>2</sub> rather than in suspension. Overall, the spectroscopic studies support the light microscopic studies and confirm the order of destruction being OM→IM→PG. Details of kinetic models of the killing mechanism are presented by Dalrymple et al. (2010).

The role of ROS in killing of bacteria is summarised in Fig. 5.

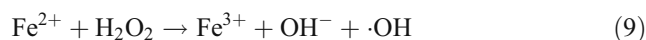
#### Role of ROS in the killing mechanism

Most studies show that ROS are responsible for the killing, and various authors propose that ·OH are responsible



**Fig. 5** Role of ROS in photocatalytic killing of bacteria. Direct oxidation of cell components can occur when cells are in direct contact with the catalyst. Hydroxyl radicals and H<sub>2</sub>O<sub>2</sub> are involved close to and distant from the catalyst, respectively. Furthermore, ·OH can be generated from reduction of metal ions, e.g. Cu<sup>2+</sup> by H<sub>2</sub>O<sub>2</sub> (Sato and Taya 2006c)

(Ireland et al. 1993; Kikuchi et al. 1997; Maness et al. 1999; Salih 2002; Cho et al. 2004, 2005; Cho and Yoon 2008). Lipid peroxidation by ROS was demonstrated by the release of MDA as a breakdown product, and there was a concurrent loss of membrane respiratory activity measured by reduction of 2,3,5-triphenyltetrazolium chloride (Maness et al. 1999). The ·OH scavengers, dimethylsulphoxide and cysteamine, eliminated the PCD activity of suspensions of TiO<sub>2</sub> in water (Salih 2002). However, ·OH are short-lived and will probably not diffuse further than 1 μm from the surface of the TiO<sub>2</sub>, especially in the presence of organic matter (Pryor 1986; Kikuchi et al. 1997). Kikuchi et al. (1997) showed that killing of *E. coli* still occurred even when the bacteria were separated from the surface by a 50-μm-thick porous membrane. However, the free radical scavenger mannitol only inhibited killing without the membrane, whereas catalase, which would degrade H<sub>2</sub>O<sub>2</sub>, decreased killing both with and without the membrane. This suggested that ·OH and H<sub>2</sub>O<sub>2</sub> were responsible for killing close to the TiO<sub>2</sub>, with H<sub>2</sub>O<sub>2</sub> acting at a distance. The role of other ROS, e.g. O<sub>2</sub><sup>·-</sup> was not considered. However, no killing was seen when cells were separated from the TiO<sub>2</sub> by a dialysis membrane in a separate study (Guillard et al. 2008). Hydrogen peroxide may act at a distance if ferrous ions are present by producing ·OH via the Fenton reaction (8 and 9).



A study of the roles of  $\text{H}_2\text{O}_2$  and  $\cdot\text{OH}$  in an immobilised  $\text{TiO}_2$  thin film reactor activated by UVC using electron spin resonance suggested that  $\cdot\text{OH}$  were produced by direct photolysis of  $\text{H}_2\text{O}_2$  as well as by Eqs. 3 and 4 (Yan et al. 2009).

A role for  $\cdot\text{OH}$  in sonocatalysis on  $\text{TiO}_2$  (where the energy to bridge the band gap is provided by sound waves) was suggested by the work of Ogino et al. 2006 who showed that the killing was inhibited by the  $\cdot\text{OH}$  scavenger glutathione. Hydroxyl radicals produced by microwave irradiation of  $\text{TiO}_2$  were shown to enhance the killing of *E. coli* (Takashima et al. 2007).

Hydroxyl radicals were shown to be the major ROS involved in killing of *C. parvum* cysts, although other ROS were also involved (Cho and Yoon 2008).

Studies with hydroxyl radical scavengers suggested that inactivation of phage in suspensions of  $\text{TiO}_2$  also occurred due to bulk phase  $\cdot\text{OH}$ , whereas inactivation of bacteria occurred with both bulk phase and surface  $\cdot\text{OH}$  (Cho et al. 2004, 2005). The rate of inactivation of *E. coli* correlated with the concentration of  $\cdot\text{OH}$ . A role for other ROS such as  $\text{H}_2\text{O}_2$  and  $\text{O}_2\cdot^-$  was also suggested.

Studies on superoxide dismutase (SOD)-defective *E. coli* have shown that oxidative damage to the membrane combined with the turgor pressure inside the cell initially permeabilizes the cell envelope, allowing critical metabolites to escape (Imlay and Fridovich 1992). Studies on oxidative damage caused by  $\text{TiO}_2$  in SOD mutants of *E. coli* showed that the inactivation rate was inversely proportional to SOD activity (Koizumi et al. 2002; Kim et al. 2004).

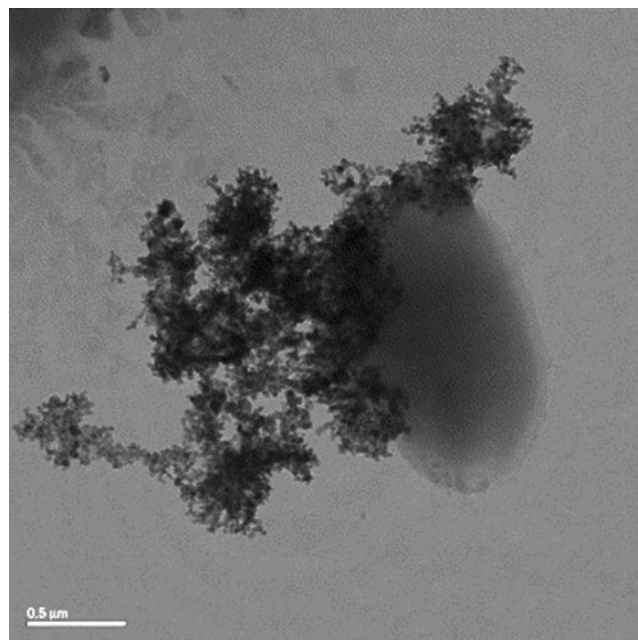
Kinetic models and further details of the chemistry of the killing mechanism are presented by Dalrymple et al. (2010). The role of  $h_{\nu b}^+$  and ROS in killing of bacteria is summarised in Fig. 5.

### Importance of contact between bacteria and $\text{TiO}_2$

Many studies have shown that close contact between the bacteria and the  $\text{TiO}_2$  increases the extent of oxidative damage. Studies on the disinfection of water have shown that suspended  $\text{TiO}_2$  is more active than  $\text{TiO}_2$  immobilised on surfaces, e.g. on thin films (Lee et al. 1997; Otaki et al. 2000; Sun et al. 2003; Gumy et al. 2006b; Marugan et al. 2006, 2008; Cohen-Yaniv et al. 2008). This is probably due to increased contact between the  $\text{TiO}_2$  particles and the bacterial cells in suspension as well as an increased surface area for ROS production. A number of studies confirm the importance of such contact (Horie et al. 1996a, b, 1998; Gumy et al. 2006a; Pratap Reddy et al. 2008; Caballero et al. 2009; Cheng et al. 2009). Co-precipitation of cells and  $\text{TiO}_2$  particles from suspension

by alum enhanced killing of *E. coli* (Salih 2004). Certain ionic species have been shown to inhibit PCD, e.g.  $\text{PO}_4^{3-}$  (Araña et al. 2002; Koizumi and Taya 2002a,b; Christensen et al. 2003; Rincón and Pulgarin 2004b; Egerton et al. 2006; Xiong et al. 2006; Marugan et al. 2008) and  $\text{HCO}_3^-$  (Rincón and Pulgarin 2004b; Coleman et al. 2005; Gogniat et al. 2006), and the rate of adsorption onto the  $\text{TiO}_2$  in the presence of different ions correlated with the rate of inactivation, suggesting that the inhibition was due to the prevention of binding of the bacteria to the  $\text{TiO}_2$  particles. Light micrographs (Nadtochenko et al. 2005; Gumy et al. 2006b; Gogniat et al. 2006) and electron micrographs clearly show binding of the titania particles to bacterial cells (Gumy et al. 2006a, b; Saito et al. 1992; Cheng et al. 2007; Shah et al. 2008). A micrograph showing particles of  $\text{TiO}_2$  attached to an *E. coli* cell is shown in Fig. 6. Contact with highly crystalline  $\text{TiO}_2$  may also cause physical damage to the cells (Liu et al. 2007c; Caballero et al. 2009).

Although differences in binding of isolated O antigens to  $\text{TiO}_2$  have been shown (*E. coli* O8 and *Citrobacter freundii* O antigens bound strongly to  $\text{TiO}_2$ , whereas that from *Stenotrophomonas maltophilia* had a low affinity for  $\text{TiO}_2$ ; Jucker et al. 1997), differences in the susceptibility of bacteria with different O antigens have not been studied. Differences in the susceptibility of different strains of *Legionella pneumophila* correlated with the amount of saturated 16C branched chain fatty acids in the membrane (Cheng et al. 2007). The more hydrophobic cells of



**Fig. 6** Transmission electron micrograph of *E. coli* showing adhesion between cells and  $\text{TiO}_2$  in suspension. Catalyst Degussa P25 pH 6.0. From Gumy et al. (2006b)

*Flavobacterium* sp. were more easily killed by PCD than *E. coli* (Cohen-Yaniv et al. 2008), which may also have been due to altered interactions with the TiO<sub>2</sub>.

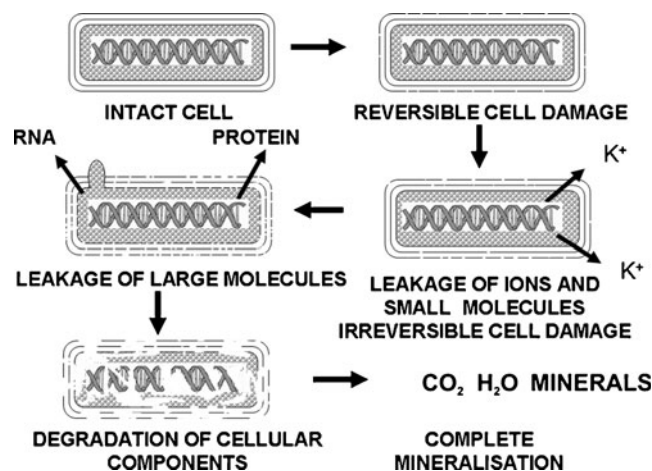
In an attempt to increase contact between the cells, Benabbou et al. (2007) studied the PCD of a strain of *E. coli* overexpressing *curli*, pili, which enhance adhesion to abiotic surfaces. However, the strain was more resistant than the non-piliated control, and evidence of protein degradation suggested that the pili were being degraded before the membrane was damaged and therefore protected the membrane from damage. The presence of extracellular polysaccharides interfered with PCD of biofilms of *P. aeruginosa* (Gage et al. 2005) and a natural biofilm (Liu et al. 2007a), but killing was seen throughout a biofilm of *Staphylococcus epidermidis* on a TiO<sub>2</sub> catalyst (Dunlop et al. 2010). The different biofilms and catalysts may explain these anomalies.

The inhibition of close contact between coliphage MS2 and TiO<sub>2</sub> by certain cations was shown by Koizumi and Taya (2002a, b), and the rate of inactivation was proportional to adsorption of the phage onto the TiO<sub>2</sub>. Sato and Taya (2006a, b) showed that the presence of organic materials protected the phage by adsorbing to the surface of the TiO<sub>2</sub>, preventing phage binding.

### Cell mineralisation

Following initial cell damage and cell death, photocatalysis has been shown to be capable of complete mineralisation of bacteria on air filters using <sup>14</sup>C-labelled cells (Jacoby et al. 1998; Wolfrum et al. 2002) and for cells suspended in water (Cooper et al. 1997; Sökmen et al. 2001). The total oxidation of *Legionella* by PCO was measured by total organic carbon analysis (Cheng et al. 2007). An almost complete degradation of *E. coli* was demonstrated on prolonged treatment on a TiO<sub>2</sub>-activated charcoal catalyst (Li et al. 2008). Nadochenko et al. (2008) showed total oxidation of cell organic matter by total internal reflection/FTIR. Removal of microorganisms during regeneration of photocatalytic TiO<sub>2</sub>-coated air filters by complete removal of contaminants has also been shown by SEM (Goswami et al. 1999; Ortiz López and Jacoby 2002). Penetration of TiO<sub>2</sub> particles into the cells was shown using an Ag/AgBr/TiO<sub>2</sub> catalyst (Hu et al. 2006).

A scheme for the killing mechanism of TiO<sub>2</sub> on bacteria is shown in Fig. 7. We suggest that there may be initial damage on contact between the cells and TiO<sub>2</sub> which affects membrane permeability, but is reversible. This is followed by increased damage to all cell wall layers, allowing leakage of small molecules such as ions. Damage at this stage may be irreversible, and this accompanies cell death. As the peptidoglycan is a highly



**Fig. 7** Scheme for photocatalytic killing and destruction of bacteria on TiO<sub>2</sub>. Contact between the cells and TiO<sub>2</sub> may affect membrane permeability, but is reversible. This is followed by increased damage to all cell wall layers, allowing leakage of small molecules such as ions. Damage at this stage may be irreversible, and this accompanies cell death. Furthermore, membrane damage allows leakage of higher molecular weight components such as proteins, which may be followed by protrusion of the cytoplasmic membrane into the surrounding medium through degraded areas of the peptidoglycan and lysis of the cell. Degradation of the internal components of the cell then occurs, followed by complete mineralisation. The degradation process may occur progressively from the side of the cell in contact with the catalyst

cross-linked molecule, damage may not be visibly evident at this stage or may be localised if the TiO<sub>2</sub> is in contact with the cells. Further membrane damage allows leakage of higher molecular weight components such as proteins. This may be followed by protrusion of the cytoplasmic membrane into the surrounding medium through degraded areas of the peptidoglycan and, eventually, lysis of the cell. Degradation of the internal components of the cell can then occur followed by complete mineralisation.

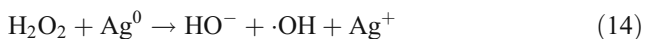
### Dual function materials

Copper-deposited films show enhanced PCD activity (Sunada et al. 2003a; Foster et al. 2010; Wu et al. 2010a; Yates et al. 2008a, b). A clear synergy in photokilling of *E. coli* on Cu-containing TiO<sub>2</sub> films was shown by Sato and Taya (2006c) who showed that H<sub>2</sub>O<sub>2</sub> was produced from the photocatalyst and Cu<sup>2+</sup> leached from the surface, but neither reached high enough concentrations to kill the *E. coli* directly. They suggested that the Cu<sup>2+</sup> was reduced to Cu<sup>+</sup> (10) which reacted with the H<sub>2</sub>O<sub>2</sub> to produce ·OH via a Fenton-type reaction (11), which was responsible for killing cells in suspension and explaining why catalase reduced this activity. Inclusion of Cu also gave higher PC activity, hence the enhanced killing of cells bound to the TiO<sub>2</sub>. In our own work, we have seen DNA damage when TiO<sub>2</sub>/

CuO surfaces were used (Fig. 4). Thus, Cu may also kill cells by DNA damage as well as membrane damage. This is consistent with the observed enhancement of damage to DNA and protein caused by ROS (Cervantes-Cervantes et al. 2005).



Similar synergy has been shown between Ag and TiO<sub>2</sub>. Ag enhances photocatalysis by enhancing charge separation at the surface of the TiO<sub>2</sub> (Sökmen et al. 2001; He et al. 2002; Hirakawa and Kamat 2005; Kubacka et al. 2008b; Liu et al. 2007b; Musil et al. 2009). Ag<sup>+</sup> is antimicrobial and can also enhance generation of ROS (Eqs. 12, 13 and 14).



## Conclusions

Generation of ROS by photocatalysis on TiO<sub>2</sub> is capable of killing a wide range of organisms including bacteria endospores in water, in air and on surfaces, including various materials. The technology has the potential to provide a powerful weapon in the fight against transmission of infectious diseases, particularly in view of the development of visible light-activated catalysts.

One of the problems is that until relatively recently, there has not been an accepted standard method for the testing of the antimicrobial efficiency of photocatalytic processes. For example, many different strains of *E. coli* have been used (Table 2) with different growth media and test conditions. This makes it very difficult to compare results from different research groups. In the second part of this review, we will investigate the evaluation of photocatalytic killing activity.

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