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ORIGINAL PAPER

Inhibition of bacteria by photocatalytic nano-TiO_2 particles in the absence of light

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Abstract The potential eco-toxicity of fourteen different nanosized titanium dioxide (TiO_2) particles was studied using Gram-positive *Bacillus subtilis* and Gram-negative *Escherichia coli* (ATCC K12) as test organisms. These photosensitive nanoparticles (NPs) were found to be harmful to the organisms studied at different degrees; the antibacterial activity increased with primary particle size, reached the maximum level in the range of 16–20 nm, and then decreased as the primary particle size increased. The presence of light played a significant role on the eco-toxicity of the nano-TiO_2 particles under most conditions studied, presumably due to the generation of reactive oxygen species (ROS). However, bacterial growth was inhibited also under dark condition, indicating that mechanisms other than photocatalytic ROS were responsible for the toxic effect. Results highlight the need for caution during the use and disposal of manufactured NPs as to prevent unintended environmental impacts, as well as the importance of further research on the mechanisms and factors that control the toxicity of NPs toward aquatic organisms.

Keywords Eco-toxicity · *Escherichia coli* (K12) · *Bacillus subtilis* · TiO_2 · Photocatalysts · Nano-particles · Particle size

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Introduction

Engineered nanoparticles (NPs) are becoming increasingly important in their applications including electronics, optics, and textiles, as well as applications in medical devices, biosensors, and in environmental remediation (Aitken et al. 2006; Handy and Shaw 2007; Handy et al. 2012). Compared to the exponential rate of increase in research on the synthesis, characterization, and applications of NPs, there was limited study on the ecotoxicological properties, behavior, impact, and fate of NPs in the aquatic environment (EU 2010). Moreover, to the best of our knowledge, there were no specific standardized protocols or certified reference materials for testing NPs. The increasing use of engineered NPs in numerous industrial applications and consumer products makes necessary to assess the risk of NPs toward human and environmental health. The behavior of NPs in the environment is not well understood. There are few available data on the toxicity of NPs on aquatic organisms. The data are often inconsistent because of biological variability and/or different test conditions. Therefore, the application of NPs can lead to many questions in environmental toxicology.

TiO_2 is a well-known antimicrobial material. It has been studied extensively over the last 25 years as photocatalyst for various purposes including the removal of organic and inorganic compounds from and the inactivation of harmful microorganism in water and air (Li et al. 2008; Caballero et al. 2009; Foster et al. 2011). Upon irradiation of Nano-TiO_2, a photocatalyst, with a photon at $\leq$385 nm wavelength, the inhibition of bacterial growth is inevitable (Matsunaga et al. 1985). A wealth of information exists on the toxicity of TiO_2 toward bacteria, mostly dealing with the photocatalytic inhibition of the bacterial growth under UV light radiation (Wei et al. 1994; Bekbolet 1997; Wamer

et al. 1997; Blake et al. 1999; Huang et al. 2000; Chai et al. 2000; Robertson et al. 2005; Coleman et al. 2005; McCullagh et al. 2007; Benabbou et al. 2007; Gibson et al. 2010; Pigeot-Rémy et al. 2011; Handy et al. 2012; Kahru and Ivask 2013). In the absence of UV irradiation, nano-TiO_2 has shown less or no effect on organisms (Dunford et al. 1997; Nakagawa et al. 1997; Rehn et al. 2003). On the other hand, several studies have reported that nano-TiO_2 causes chronic pulmonary inflammation in rats (Oberdorster et al. 1992, 2000) and proinflammatory effects in human endothelial cells (Peters et al. 2004) and bronchial epithelial cells (Gurr et al. 2005) in the absence of UV irradiation.

The results of our preliminary short-term batch and in vitro studies showed that there was also a short-term inactivation of bacteria in the absence of light (Erdem et al. 2007). Little published literature has also confirmed this finding which indicates that there are additional mechanisms responsible for the toxicity (Pagnout et al. 2012; Zhukova et al. 2012). Adams et al. (2006) showed inhibition of *Escherichia coli* and *Bacillus subtilis* by TiO_2 in dark. Gurr et al. (2005) reported from mammalian cytotoxicity studies that TiO_2 exerted oxidative stress on cells in dark under non-photocatalytic conditions.

TiO_2 is reputed to be toxic to both Gram-negative and Gram-positive bacteria. In the bacteria–TiO_2 interaction system, the Gram-positive bacteria *B. subtilis* was less sensitive to nano-TiO_2 particles than the pure culture of Gram-negative *E. coli*, possibly due to the ability of *B. subtilis* to form spores (Daughney et al. 2001; Pal et al. 2007). However, other studies have found Gram-positive bacteria to be more sensitive than Gram-negative bacteria to the antibacterial effects of TiO_2 (Yoon et al. 2007). In previous studies, the antibacterial properties of TiO_2 have been exploited in water treatment. An optimum concentration of TiO_2 in the range from 100 to 1,000 mg L^{-1} has been reported to completely disinfect the water containing 10^5–10^6 of *E. coli* cells per mL in 30 min under illuminated conditions (Wei et al. 1994; Maness et al. 1999). On the other hand, the effect of particle size of TiO_2 on inhibiting bacterial growth has not been established. The particle size of TiO_2 used on earlier studies was in the range of 20–100 μm. There was no indication whether the particle size was a key factor affecting the toxicity.

This study was to compare the toxic effects of nano-TiO_2 particles on two bacterial species, namely, Gram-negative *E. coli* and Gram-positive *B. subtilis*. The objectives were (a) to investigate the responses of bacterial population to NPs, (b) to determine the specific concentration at which TiO_2 exhibits toxic effect to the test organisms, and (c) to assess the effect of particle size on the antibacterial activity of nano-TiO_2 particles.

This work was carried out in the Aquatic Chemistry Laboratory, the Department of Civil and Environmental Engineering, University of Delaware, Newark, DE, USA, between 2008 and 2012.

Materials and methods

Culture of microorganisms

The pure cultures of Gram-negative *E. coli* K12 (ATCC 25254) and Gram-positive *B. subtilis* (ATCC 39706) were grown aerobically in 100 mL of Luria–Bertani (LB) broth on a rotary shaker (200 rpm) for 18 h at 37 and 30 °C, respectively. The cells were harvested by centrifugation at 7,800*g* for 20 min, and were suspended in 1 mL of 10 % glycerol and LB broth mixture. The stock cultures were then stored at −20 °C for further experimental uses. The final optical density of bacterial suspensions at 600 nm was determined spectrophotometrically, and the growth rates were calculated.

Nanoparticle suspensions

Table 1 gives the type, size, specific surface area, and source of nanoparticles studied. The nano-TiO_2 studied had 14 different particle diameters. Reade 5 (R5) and Reade 10 (R10) (99 % anatase) were purchased from Reade Nanostructured and Amorphous Materials Inc (Los Alamos, NM). P25 was purchased from the Degussa Corporation (80 % anatase) (Piscataway, NJ). Additional TiO_2 particles were synthesized in our laboratory by heating P25 to various temperatures in a conventional muffle for 4 h (Tseng et al. 2006; Dong et al. 2007). The nano-Fe particles were provided by Professor Mao Hong Fan (University of Wyoming). The γ-Al_2O_3 particles were purchased from the Allied High Tech Products (Rancho Dominguez, CA).

The powder X-ray diffraction (Rigaku D Max B) with a CuKα radiation source was used to verify the crystal phase. The transmission electron microscopy (TEM, Jeol 2000) and dynamic light scattering (DLS, Malvern 3000HS, Malvern, PA) techniques were used to determine the particle size. The specific surface area was obtained using N_2-gas adsorption following the Brunauer–Emmett–Teller equation, i.e., the BET method (Nova 2000, Quantachrome Corp., Boynton, FL).

Stock suspensions (1,000 mg L^{-1}) were prepared with LB broth (171 mM) and Min salt media (71 mM), and the diluted TiO_2 suspensions were added to aliquots of the culture media immediately prior to experiments. The final TiO_2 concentration was in the range of 0.01 and 1,000 mg L^{-1}. All experiments were conducted in continuously shaken aqueous slurry solutions to ensure mixing and prevent settling of NPs.

Table 1 Nanoparticles used in the study

Name	Diameter[a] (nm)	Surface area (BET, m^2 g^{-1})	Size calculation method	Manufacturing method	Source
UV100	4.7	329.2	XRD, TEM	Sol–gel	Sachtleben Chem. http://www.sachtleben.com/
R10	4.8	319.3	XRD, SEM	Wet-chemistry precipitation	Reade Materials
R5	5.2	294.6			http://www.reade.com/
W_o6	12.3	125.4	SEM, DLS	Reverse micelle	Dong et al. (2007)
T1	16.2	95.0			
T6	17.8	86.5			
W_o3	21.4	72.0			
S3	27.5	55.9	TEM	Sol–gel	
S2	30.3	50.9			
P25	34.2	45.1	TEM	Liquid–gas phase synthesis	Degussa-Evonik http://www.evonik.com/
$Y10_{600}$	42.0	33.6	TEM	Temperature annealing	Tseng et al. (2006)
$Y7_{660}$	45.8	13.8			
$Y9_{780}$	120.1	12.8			
$Y8_{840}$	204.1	7.5			
γ-Al_2O_3	15.0	103.0	TEM	Liquid–gas phase synthesis	Degussa-Evonik http://www.evonik.com
Nano-Fe[a]	40.0	30.0	TEM	Sol gel	Prof. Fan

[a] Courtesy of Professor Mao Hong Fan, University of Wyoming

Cell viability

Direct colony counting technique was used to determine the cell viability. The number of viable cells in suspensions that were subjected to the dark treatments was determined by plating serially diluted suspensions (100 μL) onto LB agar plates. The plates were incubated at 37 °C (*E. coli*) and 30 °C (*B. subtilis*) for 24 h. Then the number of colonies on the plates was counted using Fisher Acculite 133-8002 model colony counter. The cell viability runs were conducted in triplicates.

Experimental setup

Two levels of bacterial population density, high ($>10^9$ CFU mL^{-1}) and low ($<10^5$ CFU mL^{-1}), were used in nine separate runs (Table 2). Two different media were studied, a high ionic strength of LB Broth (171 mM) and a low ionic concentration of Min salt (71 mM). A constant speed of 200 rpm was used to maintain an aerobic condition in the system. The experiments were conducted at room temperature (23 ± 0.8 °C). Freshly prepared NP suspensions from stocks were added to the bacteria–media mixture immediately prior to experiments.

Results and discussion

General microbial responses: (phases and initial population density)

Gram-negative *E. coli* and Gram-positive *B. subtilis* are the most investigated species in toxicity studies (Pal et al. 2007). For purpose of comparison with published results in the literature, *E. coli* K12 and *B. subtilis* were selected. The effect of initial cell population on cell inhibition was not well reported to this date. The inhibition of total coliforms has often been shown to be of the first-order kinetics (Watts et al. 1995; Li et al. 1996). When the cell population was $\leq 10^3$ CFU mL^{-1}, the inhibition rate was independent of the initial cell concentration (Li et al. 1996). The inhibition of *B. pumilus* was increased with initial cell concentration between 10^4 and 10^9 CFU mL^{-1} (Huang et al. 2000). No results are available on the effect of initial cell population on the rate of cell death, however.

In order to understand the general microbial growth responses to nano-TiO_2 (P25) under dark condition, a series of diluted *E. coli* concentrations were studied (Fig. 1). *E. coli* cultures, with an optical density of 1.0 at 600 nm, were serially diluted with Min salt media. The initial population numbers (N_o) and that after 60-min exposure

Table 2 Experimental design and conditions

Run (#)	Culture	Media	Initial concentration (CFU/mL)	Instrument	Speed (rpm)	Incubation
1	*E. coli*	LB broth (170 mM)	2×10^9	Jacketed beaker	Max stirring	37 °C, 24 h
2			2×10^9	Bench shaker	200	
3			2×10^5			
4	*E. coli*	Min salt (70 mM)	10^9	Floor shaker	200	37 °C, 24 h
5			10^3			
6			10^9	Bench shaker		
7			10^3			
8	*B. subtilis*	Min salt (70 mM)	10^9	Bench shaker	200	30 °C, 24 h
9	*B. subtilis*		10^3			

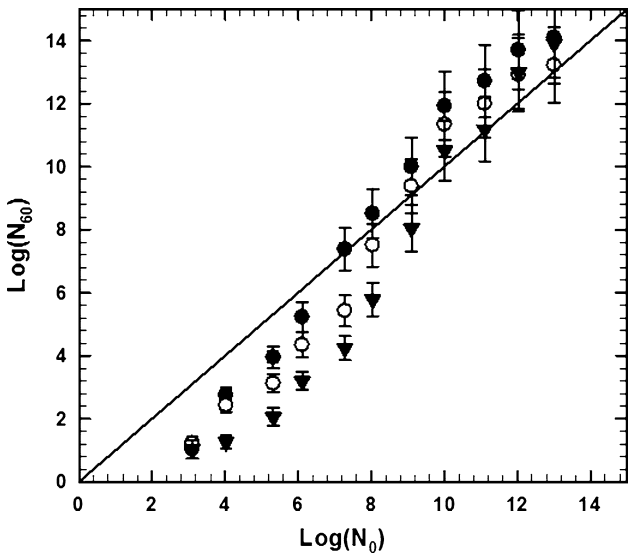

Fig. 1 Effect of initial bacterial population density on the growth/die-off of *E. coli* in the presence of TiO_2 under dark condition. Experimental condition: P25 TiO_2 concentration (in LB Broth media): (*black circle*) 10 mg L^{-1}, (*white circle*) 100 mg L^{-1} and (*black down pointing triangle*) 1,000 mg L^{-1} TiO_2); exposure time = 1 h; bench shaker mixing rate = 200 rpm; room temperature; number of experiments = triplicate

(N_{60}) were obtained by direct colony counting. Results in Fig. 1 show that bacteria kill when the initial population was $\leq 10^5$, $\leq 10^8$, and $\leq 10^9$ CFU/mL in the presence of TiO_2 at concentration of 10, 100, and 1,000 mg L^{-1}, respectively. Likewise, there was net population growth when the TiO_2 concentration was $\geq 10^5$, $\geq 10^8$, and $\geq 10^9$ CFU/mL in the presence of TiO_2 at concentration of 10, 100, and 1,000 mg L^{-1}, respectively. It appeared that higher bacterial population was able to sustain the inhibitory effect of the nano-TiO_2 particles. For example, at high TiO_2 concentration, i.e., >1,000 mg L^{-1}, the initial population above which positive bacterial growth occurred was 10^9 CFU/mL versus 10^5 CFU/mL in the presence of 10 mg L^{-1} of TiO_2. This observation is crucial to the design of antibacterial process using photocatalytic TiO_2.

Figure 2 shows the responses *E. coli* cells to nano-TiO_2 under dark condition as affected by initial population density. A low concentration (4.3×10^5 CFU/mL) and a high concentration (3.1×10^9 CFU/mL) of *E. coli* K12 cultures were employed both under dark condition at room temperature (23 ± 0.8 °C) for the purpose of comparison. TiO_2 (100 mg L^{-1} in concentration and 16.2 nm in size) was added to the growth media. The standard deviations were calculated from experiments run in triplicate as the error bars shown in the figure. Figure 2 shows that there was a bi-phasic die-off in *E. coli* when the initial population was low. A rapid decrease in population occurred within the first 10 min followed by a slow inhibition after 15 min, which can be attributed to the lag-growth phase. Data, not presented, indicated that as the exposure time was longer than 1 h, there was an increase in the cell density over the control (Erdem 2008). The bi-phasic die-off of *E. coli* was absent in the control when the initial population was $>10^9$ CFU/mL.

Effect of nanoparticle concentration

Five different TiO_2 concentrations ranging from 0.1 to 1,000 mg L^{-1} and 14 different particle sizes ranging from 4.8 to 204.1 nm were studied to examine changes in cellular viability resulting from the biocidal action of TiO_2. In order to understand the killing mechanism of TiO_2, a Gram-positive species, *B. subtilis*, and a Gram-negative species, *E. coli* K12, were employed in the study. It was hypothesized that Gram-negative cells having an outer cellular membrane may have additional protection against physical and chemical damages.

Figure 3 shows the effect of the concentration of P25 TiO_2 on the die-off of *E. coli* K12 and *B. subtilis*. As the particle concentration increased, the degree of bacterial die-off increased to a plateau then decreased with further increase in TiO_2 concentration. Low TiO_2 concentration, between 0.1 and 10 mg L^{-1}, was not as effective as that of high concentrations (e.g., 100–1,000 mg L^{-1}) in terms of

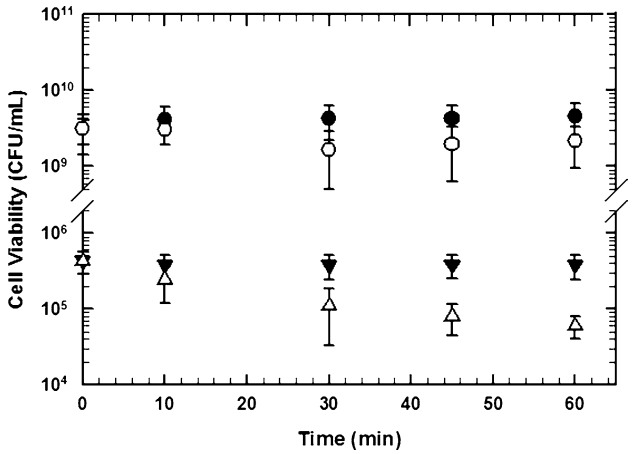

Fig. 2 Effect of the initial population density on the die-off of *E. coli* in the absence and presence of TiO_2 nanoparticles (100 mg L^{-1} at 16 nm in diameter) under dark condition. Experimental conditions: TiO_2 concentration: (*black circle*) high bacterial population density (*E. coli* only), (*white circle*) high bacterial population density (*E. coli* with TiO_2), (*black down pointing triangle*) low bacterial population density (*E. coli* only), () low bacterial population density (*E. coli* with TiO_2)

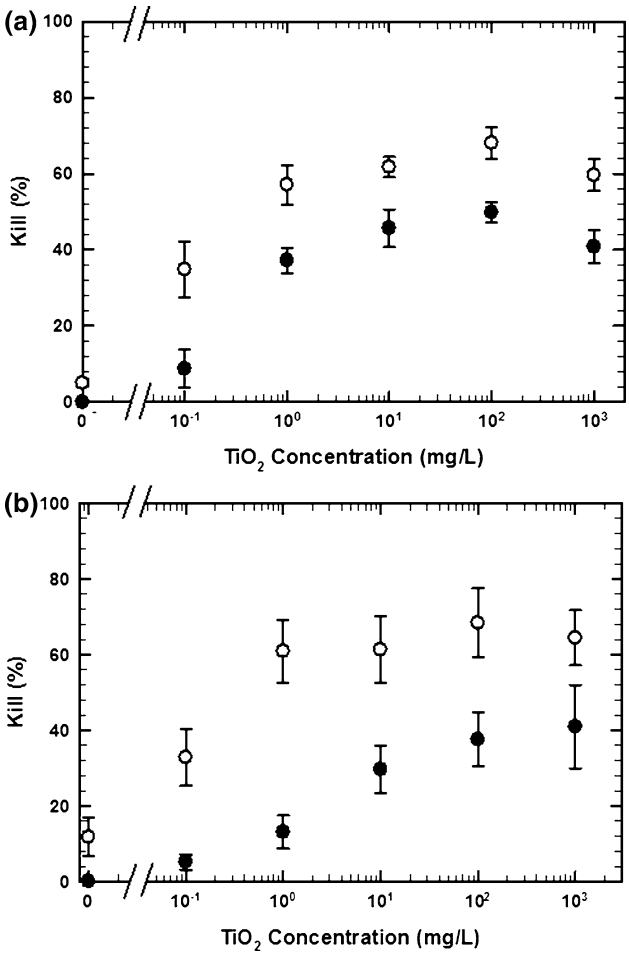

Fig. 3 Effect of P25 TiO_2 particle concentration on the die-off of *E. coli* (**a**) and *B. subtilis* (**b**) under dark condition. Experimental condition: exposure time = (*black circle*) 10 min, (*white circle*) = 60 min; bench shaker mixing rate = 200 rpm; room temperature; number of experiment = triplicate

bacterial die-off. According to Fig. 3a, when the particle concentration was 100 mg L^{-1}, P25 TiO_2 exhibited the most significant effect on the die-off of both *E. coli* and *B. subtilis,* whereas at the highest concentration of 1,000 mg L^{-1}, it was more damaging to the *B. subtilis* under the same exposure time (Fig. 3b). The Toxicity Relationship Analysis Program version 1 (TRAP) was applied to calculate the effective concentration for 50 % kill of the *E. coli* (i.e., LC50) at 10 and 60 min, respectively. The results showed that the LC50 was 308 and 126 mg L^{-1} at exposure time of 10 and 60 min, respectively. The data shown in the Fig. 3b were taken at 10 and 60 min. P25 TiO_2 (0.1–1,000 mg L^{-1}) was used in Min salt media. Based on the TRAP program, the LC50 were 509 and 428 mg L^{-1} at 10 and 60 min, respectively. The results showed that *E. coli* K12 cells were more sensitive to TiO_2 than *B. subtilis*. The difference in the cell morphology would render *B. subtilis* more resistant to NPs than *E. coli*. Adams et al. (2006) studied the effect of TiO_2 on *E. coli* and *B. subtilis* in the presence and absence of light. The TiO_2 concentration between 10 and 5,000 mg L^{-1} was used. Results showed that the EC50 were 3,150 and 835 mg L^{-1} for for *E. coli* and *B. subtilis*, respectively, for TiO_2 (330 nm).

Effect of particle size

TiO_2 in three different particle sizes, 5.2, 6.5, and 34.2 nm were used to assess the effect of particle size on the survival of *E. coli* (9.3×10^9 CFU/mL) under dark condition in 71 mM Min salt media. Figure 4a shows the effect of particle size on the population density of *E. coli*. The control set showed almost no decrease in population growth, which could be attributed to the high cell concentration. In 10 min, 2.5-log bacterial die-off occurred rapidly. The effect of particle size at 45 min of exposure showed no statistical difference ($p > 0.01$), however.

Figure 4b shows that the die-off of *E. coli* followed the two-stage kinetics, a fast first stage in the first 10 min followed by a slower second stage after 10 min. The rate constant of stage 1, k_1, was greater than that of stage 2, k_2.

A much detailed demonstration of the effect of particle size on the responses of bacteria can be seen in Fig. 5. *E. coli* K12 and *B. subtilis* were treated with 100 mg L^{-1} of TiO_2 for 60 min. *E. coli* (3.6×10^6 CFU/mL), and *B. subtilis* (1.3×10^6 CFU/mL) at low population were grown in Min salt (71 mM) media. The results indicated

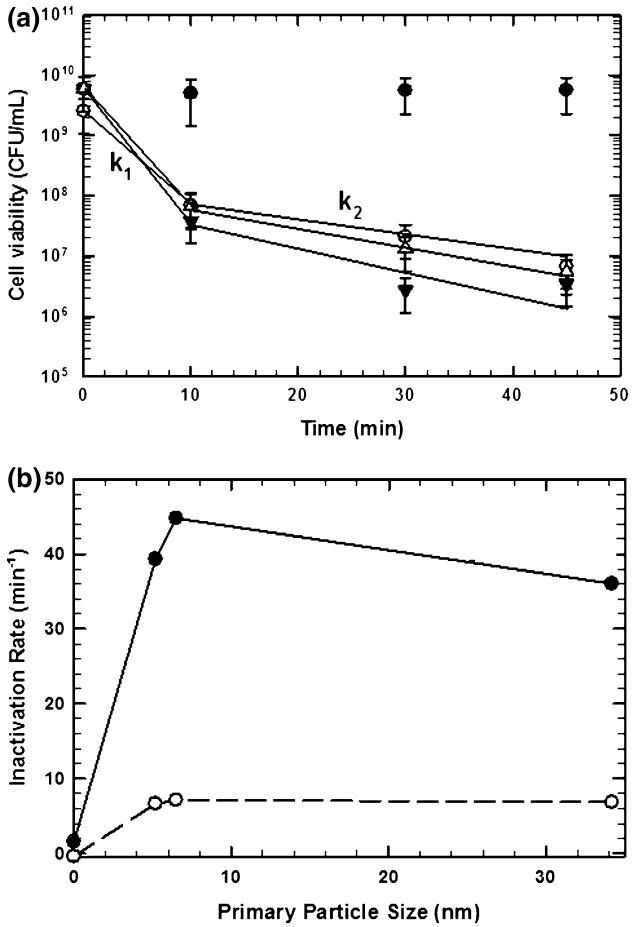

Fig. 4 Kinetics of the survival of *E. coli* under dark condition in the presence of TiO_2 (100 mg L^{-1}). **a** population density as a function of exposure time: (*black circle*) control, (*white circle*) 5.2 nm, (*black down pointing triangle*) 6.5 nm, () 34.2 nm); **b** die-off rate constant as a function of primary particle size: (*black circle*) k_1, (*white circle*) k_2

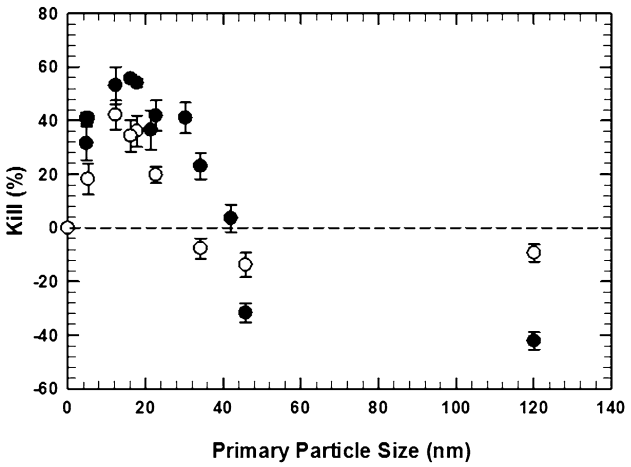

Fig. 5 Effect of primary particle size on the survival of *E. coli* and *B. subtilis* under dark condition in the presence of TiO_2 (100 mg L^{-1}). (*black circle*) *E. coli*, (*white circle*) *B. subtilis*

that under dark condition, particle size at 18 nm was the most biocidal for both bacteria. When the primary particle size was larger than 35 and 40 nm, there was no significant inhibitory effect on the growth of *B. subtilis* and *E. coli* bacteria, however. It is noted that at particle size greater than 35–40 nm, the bacterial population increased. At particle diameter >35–40 nm, the population of *E. coli* and *B. subtilis* was increased by 10 and 40 %, respectively. Results have important implication in the application of nanoparticles as antibacterial agent that there exists an optimal particle size at which nanoparticles exhibits the maximum bacterial kills.

Since there was no irradiation in the system, no ROS attack might occur. Therefore, a possible explanation to the bacterial inhibition could be nutrient uptake or waste disposal interruption by the presence of nanoparticles. It is well known that most nanoparticles are electrically charged in the aquatic environment due to various surface-charging mechanisms (Stumm and Morgan 1996); surface charge enables their adsorption onto the cellular membrane. This may result in blocking the transport of nutrients and metabolic wastes in and out of cells.

Effect of different type of NPs

In order to compare the population response of *E. coli* to different NPs, TiO_2, nano-Fe, and γ-Al_2O_3 at concentration of 100 mg L^{-1} were studied under dark condition. TiO_2 in three different particle sizes of 4.8 nm (specific surface area = 319 m^2 g^{-1}), 16.2 nm (95 m^2 g^{-1}), and 34.2 nm (45 m^2 g^{-1}) were chosen to compare the impacts to bacteria. The particle size of nano-Fe NPs and γ-Al_2O_3 was 40 (specific surface area = 30 m^2 g^{-1}) and 15.8 nm (specific surface area = 103 m^2 g^{-1}), respectively. The specific die-off rate constant (k') at 60 min was used to quantify the antimicrobial activities of nanoparticles (Table 1). The specific die-off constant, k′ (L mg^{-1}), is defined by Eq. 1 as follows:

$$k' = -\frac{\ln(N_t/N_o)}{C} \quad (1)$$

where N_t is the bacterial population (CFU/mL) at a given time, N_o is the initial population (CFU/mL), and C is the concentration of NPs (mg L^{-1}). A larger k' value means that the bacteria are more sensitive to the NPs, indicating more antimicrobial activity of the NPs (Yoon et al. 2007).

Table 3 shows that TiO_2 expressed the lowest biocidal effect to the *E. coli* among all nanoparticles studied and

Table 3 Summary of specific die-off rate of *E. coli* under dark condition in the presence of different nanoparticles

Time (min)	Specific die-off rate (10^{-2} L/(mg min)				
	γ-Al_2O_3	Nano-Fe	TiO_2		
	15 nm	40 nm	4.8 nm	16.2 nm	34.2 nm
10	4.98	5.67	1.41	1.28	1.39
60	4.48	5.26	1.23	0.52	1.11

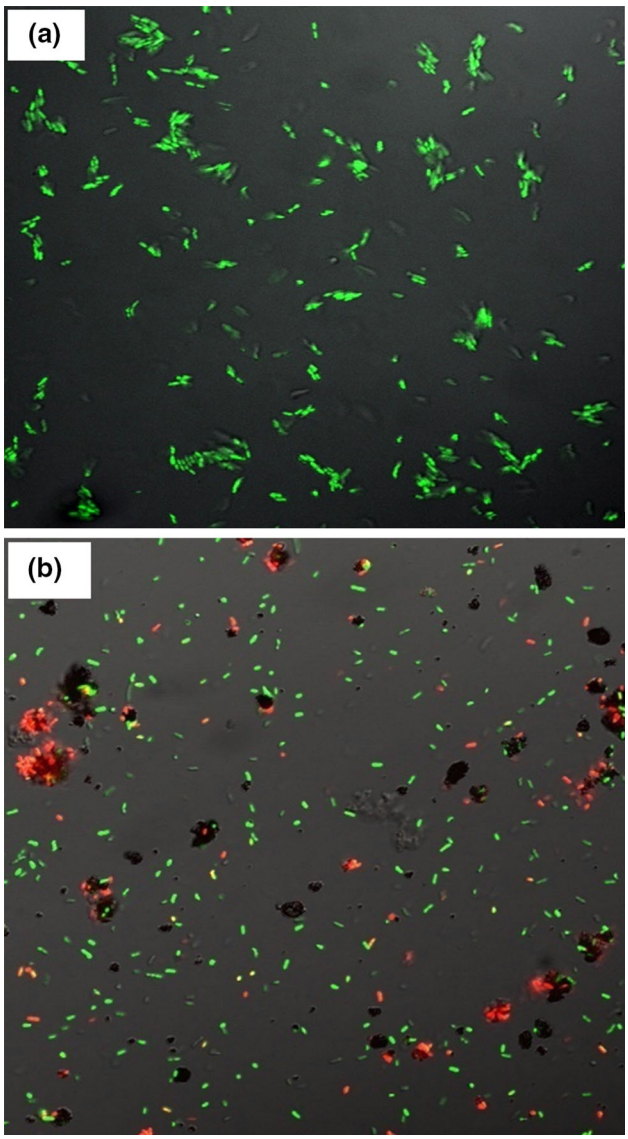

Fig. 6 Confocal image of *E. coli* in the absence (**a**) and the presence of TiO_2 (100 mg L^{-1} P25)

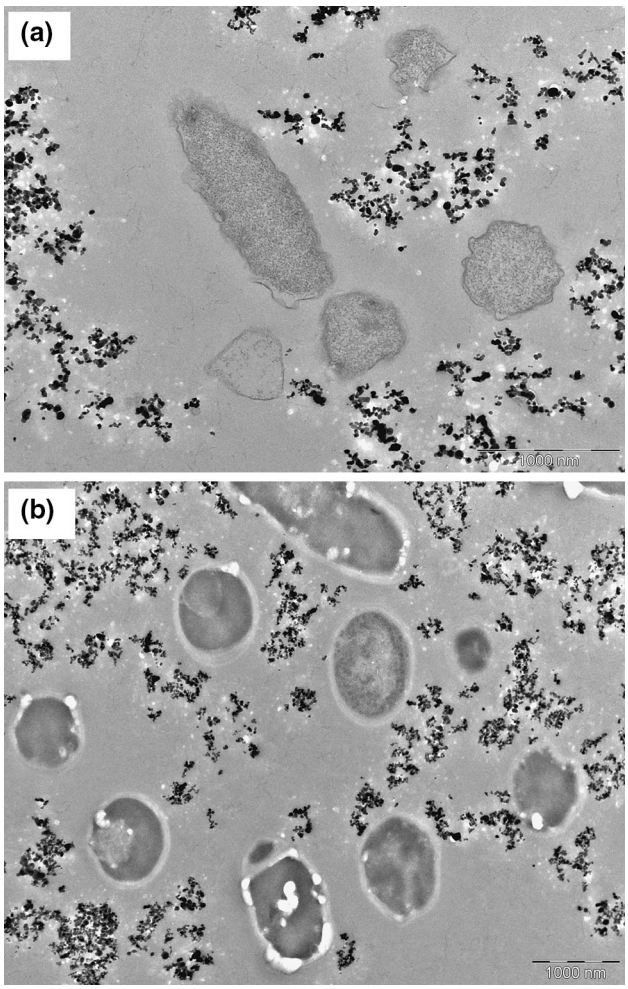

Fig. 7 TEM image of *E. coli* in the absence (**a**) and the presence of TiO_2 (100 mg L^{-1} P25)

that there was a critical primary particle size that exhibits the maximum bacterial kill. When all three types of NPs were compared, the toxicity followed the increasing order: TiO_2 (16.2 nm) < TiO_2 (34.2 nm) ~ TiO_2 (4.8 nm) < γ-Al_2O_3 (15.8 nm) < Nano-Fe (40 nm).

Preliminarily proposed killing mechanisms

One possible killing mechanism under dark condition would be the interaction between the NPs and bacteria. The bacteria have a small size; however, it has a very large surface area: volume ratio, which makes possible the fast transfer of nutrients and waste materials between the

interior of the cell and its environment. Nikaido et al. (1980) explained that diffusion channels in the outer membrane of intact cells, namely *E. coli*, had a diameter between 0.6 and 1.2 nm. In an early study, Steven et al. (1977) showed that an *E. coli* porin revealed indentations with a diameter of 1.5–2 nm. When the NPs and bacteria are in contact, the NPs may plug the pores of the diffusion channels and prevent nutrition uptake.

Figure 6 shows the confocal images of the control *E. coli* (Fig. 6a) and bacteria treated with 100 mg L^{-1} of P25 TiO_2 under dark condition (Fig. 6b). Figure 6a clearly shows the healthy bacteria cells reflecting the green fluorescent color and some dead bacteria in red color. The agglomerated TiO_2–bacteria clusters can be seen in Fig. 6b. The dead bacteria had surface characteristics that enhanced cell attachments to either bacterial cells or nanoparticles and formed clusters.

Figure 7 shows the TEM images of *E. coli* (Fig. 7a) and *B. subtilis* (Fig. 7b) treated with 100 mg L^{-1} of P25 under dark condition. It can clearly be seen that the outer cell membrane of *E. coli* was damaged (Fig. 7b); however, the attachment of NPs and bacteria was not observed. The physical change in *B. subtilis* (Fig. 7b) was not as visible as that of *E. coli*. It has been showed that Gram-positive *B. subtilis* are more resistant to the stressor than Gram-negative *E. coli*.

Conclusion

The presence of NPs has adverse effect on the growth of bacteria, especially at low cell population density, e.g., $\leq 10^5$ CFU/mL when adverse effect was easily detectable. A high population density, e.g., $\geq 10^9$ CFU/mL would lead to a faster bacterial growth and decreased the antimicrobial effect of NPs.

When the ionic strength of the growth media increased, the bacterial growth rate became greater than the die-off rate. With the presently available information, TiO_2 exhibited larger biocidal activity toward *E. coli* than nano-Al_2O_3 and nano-Fe particles. The difference in biocidal activity might be attributed in part to the degree of attachment between the NPs and the bacteria. The pH_{zpc} of Al_2O_3 was 9.0 and that of TiO_2 was 5.5. The higher negative surface charge of Al_2O_3 could increase the adsorption of NPs onto the negatively charged bacteria surface.

The die-off of bacteria takes place in two stages: fast first and slow second. The higher particle concentration and smaller primary particle size appeared to be more damaging to the bacteria under dark condition. There was a crucial primary particle size that induced the highest degree of bacterial killings; results showed that 18 nm was the most antimicrobial particle size. This study is the first to examine the effect of primary particle size on the survival of bacteria exemplified by TiO_2 nanoparticles. Finally, TEM and confocal images showed that when bacteria were treated with TiO_2 NPs, the morphology of the cell structure changed.

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References

Adams LK, Lyon DY, Alvarez PJJ (2006) Comparative eco-toxicity of nanoscale TiO_2, SiO_2, and ZnO water suspensions. Water Res 40:3527–3532

Aitken RJ, Chaudhry MQ, Boxall ABA, Hull M (2006) Manufacture and use of nanomaterials: current status in the UK and global trends. Occup Med 56(5):300–306

Bekbolet M (1997) Photocatalytic bactericidal activity of TiO_2 in aqueous suspensions of *E. coli*. Water Sci Technol 35(11–12):95–100

Benabbou AK, Derriche Z, Felix C, Lejeune P, Guillard C (2007) Photocatalytic inactivation of *Escherischia coli* effect of concentration of TiO_2 and microorganism, nature, and intensity of UV irradiation. Appl Catal B 76(3–4):257–263

Blake DM, Maness PC, Huang Z, Wolfrum EJ, Huang J, Jacoby WA (1999) Application of the photocatalytic chemistry of titanium dioxide to disinfection and the killing of cancer cells. Sep Purif Methods 28(1):1–50

Caballero L, Whitehead KA, Allen NS, Verran J (2009) Inactivation of *Escherichia coli* on immobilized TiO_2 using fluorescent light. J Photochem Photobiol A Chem 202(2–3):92–98

Chai YS, Lee JC, Kim BW (2000) Photocatalytic disinfection of *E-coli* in a suspended TiO_2/UV reactor. Korean J Chem Eng 17(6):633–637

Coleman HM, Marquis CP, Scott JA, Chin SS, Amal R (2005) Bactericidal effects of titanium dioxide-based photocatalysts. Chem Eng J 113(1):55–63

Daughney CJ, Fowle DA, Fortin DE (2001) The effect of growth phase on proton and metal adsorption by *Bacillus subtilis*. Geochim Cosmochim Acta 61:1025–1035

Dong CD, Liao YL, Kao CM, Chen CW, Lin HY, Huang CP (2007) Preparation of crystalline nanosized titania by microemulsion: evaluation of process variables. J Adv Oxid Technol 10(2):399–404

Dunford R, Salinaro A, Cai L, Serpone N, Horikoshi S, Hidaka H, Knowland J (1997) Chemical oxidation and DNA damage catalysed by inorganic sunscreen ingredients. FEBS Lett 418(1/2):87–90

Erdem A (2008) Short-term toxicity of photocatalytic titainum dioxide to bacteria under ambient conditions. University of Delaware, Newark

Erdem A, Cha D, Huang CP (2007) Chapter 9: growth and some enzymatic responses of *E. coli* to photocatalytic TiO_2. In: Grassian VH (ed) Nanoscience and nanotechnology: environmental and health impact. Wiley, Hoboken, p 496

EU (2010) European Union. In: Riediker MAK, Lausanne G (eds) Compendium of projects in the european nanosafety cluster. Switzerland, Institute for Work and Health, p 196

Foster HA, Ditta IB, Varghese S (2011) Photocatalytic disinfection using titanium dioxide: spectrum and mechanism of antimicrobial activity. Appl Microbiol Biotechnol 90:1847–1868

Gibson RM, Adisesh A, Bergamaschi E, Berges M, Bloch D, Hankin S, Lynch I, Riediker M (2010) Strategies for assessing occupational health effects of engineered nanomaterials. Institute for Work and Health, Lausanne, Switzerland, p 27

Gurr JR, Wang ASS, Chen CH, Jan KY (2005) Ultrafine titanium dioxide particles in the absence of photoactivation can induce oxidative damage to human bronchial epithelial cells. Toxicology 213(1–2):66–73

Handy RD, Shaw BJ (2007) Toxic effects of nanoparticles and nanomaterials: implications for public health, risk assessment and the public perception of nanotechnology. Health Risk Soc 9(2):125–144

Handy R, Cornelis G, Fernandes T, Tsyusko O, Decho A, Sabo-Attwood T, Metcalfe C, Steevens JA, Klaine SJ, Koelmans AA, Horne N (2012) Ecotoxicity test methods for engineered nanomaterials: practical experiences and recommendations from the bench. Environ Toxicol Chem 31(1):15–31

Huang Z, Maness P-C, Blake DM, Wolfrum EJ, Smolinski SL, Jacoby WA (2000) Bactericidal mode of titanium dioxide photocatalysis. J Photochem Photobiol A Chem 130:163–170

Kahru A, Ivask A (2013) Mapping the dawn of nanoecotoxicological research. Acc Chem Res 46(3):823–833

Li XZ, Zhang M, Chua H (1996) Disinfection of municipal wastewater by sensitized photooxidation. Water Sci Technol 33(3):111–118

Li QL, Mahendra S, Lyon DY, Brunet L, Liga MV, Li D, Alvarez PJJ (2008) Antimicrobial nanomaterials for water disinfection and microbial control: potential applications and implications. Water Res 42(18):4591–4602

Maness PC, Smolinski S, Blake DM, Huang Z, Wolfrum EJ, Jacoby WA (1999) Bactericidal activity of photocatalytic TiO_2 reaction: toward an understanding of its killing mechanism. Appl Environ Microbiol 65(9):4094–4098

Matsunaga T, Tomoda R, Nakajima T, Wake H (1985) Photoelectrochemical sterilization of microbial-cells by semiconductor powders. FEMS Microbiol Lett 29(1–2):211–214

McCullagh C, Robertson JMC, Bahnemann DW, Robertson PKJ (2007) The application of TiO_2 photocatalysis for disinfection of water contaminated with pathogenic micro-organisms: a review. Res Chem Intermed 33(3–5):359–375

Nakagawa Y, Wakuri S, Sakamoto K, Tanaka R (1997) The photogenotoxicity of titanium dioxide particles. Mutat Res 394:125–132

Nikaido H, Luckey M, Rosenberg EY (1980) Nonspecific and specific diffusion channels in the outer membrane of *Escherichia coli*. J Supramol Struct 13(3):305–313

Oberdorster G, Ferin J, Gelein R, Soderholm SC, Finkelstein J (1992) Role of the alveolar macrophage in lung injury: studies with ultrafine particles. Environ Health Perspect 97:193–199

Oberdorster G, Finkelstein JN, Johnston C, Gelein R, Cox C, Baggs R, Elder AC (2000) Acute pulmonary effects of ultrafine particles in rats and mice. Res Rep Health Eff Inst 96:5–74

Pagnout C, Jomini S, Dadhwal M, Caillet C, Thomas F, Bauda P (2012) Role of electrostatic interactions in the toxicity of titanium dioxide nanoparticles toward *Escherichia coli*. Colloids Surf B 92:315–321

Pal A, Pehkonen SO, Yu LE, Ray MB (2007) Photocatalytic inactivation of gram-positive and gram-negative bacteria using fluorescent light. J Photochem Photobiol A Chem 186(2–3):335–341

Peters K, Unger RE, Kirkpatrick CJ, Gatti AM, Monari E (2004) Effects of nano-scaled particles on endothelial cell function in vitro: studies on viability, proliferation and inflammation. J Mater Sci Mater Med 15:321–325

Pigeot-Rémy S, Simonet F, Errazuriz-Cerda E, Lazzaroni JC, Atlan D, Guillard C (2011) Photocatalysis and disinfection of water: identification of potential bacterial targets. Appl Catal B 104(3–4):390–398

Rehn B, Seiler F, Rehn S, Bruch J, Maier M (2003) Investigations on the inflammatory and genotoxic lung effects of two types of titanium dioxide: untreated and surface treated. Toxicol Appl Pharmacol 189(2):84–95

Robertson JMC, Robertson PKJ, Lawton LA (2005) A comparison of the effectiveness of TiO_2 photocatalysis and UVA photolysis for the destruction of three pathogenic micro-organisms. J Photochem Photobiol A Chem 175(1):51–56

Steven AC, ten Heggeler B, Muller R, Kistler J, Rosenbusch JP (1977) Ultrastructure of a periodic protein layer in the outer membrane of *Escherichia coli*. J Cell Biol 72(2):292–301

Stumm W, Morgan JJ (1996) Aquatic chemistry: chemical equilibria and rates in natural waters. Wiley, New York

Tseng YH, Lin HY, Kuoc CS, Lic YY, Huang CP (2006) Thermostability of nano-TiO_2 and its photocatalytic activity. React Kinet Catal Lett 89(1):63–69

Wamer WG, Yin JJ, Wei RR (1997) Oxidative damage to nucleic acids photosensitized by titanium dioxide. Free Radic Biol Med 23(6):851–858

Watts RJ, Kong S, Orr MP, Miller GC, Henry BE (1995) Photocatalytic inactivation of coliform bacteria and viruses in secondary wastewater effluent. Water Res 29(1):95–104

Wei C, Lin WY, Zainal Z, Williams NE, Zhu K, Kruzic AP, Smith RL, Rajeshwar K (1994) Bactericidal activity of TiO_2 photocatalyst in aqueous-media—toward a solar-assisted

water disinfection system. Environ Sci Technol 28(5):934–938

Yoon KY, Byeon JH, Park JH, Hwang J (2007) Susceptibility constants of *Escherichia coli* and *Bacillus subtilis* to silver and copper nanoparticles. Sci Total Environ 373:572–575

Zhukova LV, Kiwi J, Nikandrov VV (2012) TiO_2 nanoparticles suppress *Escherichia coli* cell division in the absence of UV irradiation in acidic conditions. Colloids Surf B 97:240–247