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ZnO nanoparticles in complete photo-mineralization of aqueous gram negative bacteria and their organic content with direct solar light

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ABSTRACT

For the first time, pristine ZnO nano-particles can be used as effective catalyst for water disinfection by killing and complete mineralization of two gram negative bacteria with direct solar light. Just like in earlier studies, pristine nano-size ZnO particles have shown anti-bacterial activity against two types of gram negative bacteria, *E. coli* and *P. aeruginosa*, where up to 20% of the former and 25% of the latter have been killed in the dark. Under direct solar radiation, the pristine ZnO particles readily catalyzed bacterial photo-degradation. While earlier studies were mostly limited to bacterial death and growth inhibition by pristine ZnO particles, the results describe for the first time how bacteria and their organic content can be completely photo-mineralized by direct solar radiations in 60 min. Only the bacterial cell wall fragments resisted the photo-degradation process. Under the reaction conditions, the degradation occurred by the UV tail of the direct solar light, where the ZnO nano-particles behaved as photo-catalysts. The results show the added value of using ZnO nano-particles as photo-catalysts in water disinfection strategies, leaving no resulting organic molecules in water.

1. Introduction

Continued contamination is making fresh healthy water a challenge, as only about 1.1% of natural waters are safe to drink [1]. Water is contaminated by different chemical and biological contaminants. Biological contamination by different types of pathogenic bacteria, protozoa and viruses, is a real threat to human beings causing different diseases [2]. Lack of control on human wastes is one serious cause for contamination [3]. Drinking water contamination with different types of bacteria is documented [2]. One class of widely known bacteria is *E. coli,* which belongs to gram-negative rod-shaped bacteria, with typical dimensions of ~2 μm in length and ~0.5 μm in width. *E. coli* may be hazardous (causing diarrhea and gastroenteritis) or harmless, depending on their types [4–6]. *P. aeruginosa* bacteria are other gram negative bacteria capable of causing serious infections in insects, plants and animals. *P. aeruginosa* is a major cause of nosocomial infections and causes chronic lung infections that affect cystic fibrosis [7]. *P. aeruginosa* is well known for its resistance to anti-bacterial agents [8]. Both bacteria are hazardous and should thus be completely removed from drinking waters.

Disinfecting drinking waters is a globally common practice. Different methods of disinfection are being used. Chlorination (with Cl_2, NaOCl, $Ca(OCl)_2$, chloramines or chlorine dioxide) is commonly used, but causes the production of chlorinated organic compounds in the municipality drains [9,10]. Other disinfection methods, are also used such as UV radiation and ozonolysis [11]. Such methods are effective but could be costly if used at large scale processes.

Nano-particles have been widely described as tools to kill micro-organisms [12–14]. Huang et al. reported that "*Cell wall damage, followed by cytoplasmic membrane damage, leading to a direct intracellular attack, has therefore been proposed as the sequence of events when microorganisms undergo TiO_2 photo-catalytic attack*" [15]. ZnO nanopowders have been reported for killing *E. coli*, *S. aureus* and *Bacillus atrophaeus* [16,17], although Adams *et al.* ruled out any effect for ZnO nano-particles against *E. coli* [18]. Zhang reported that ZnO particles exhibited activity with certain sizes [19,20]. The effect of

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pH on ZnO activity against bacteria was also reported [21]. Effect of ZnO particle storage, under different conditions, on their antibacterial activity against *E. coli* was investigated by Zhang et al. [20]. Doping the ZnO particles with other elements further enhanced their antibacterial activity [14].

Bacterial death and growth inhibition are thus well documented. A mechanism to explain the effect of ZnO particles on bacteria, based on electrostatic interactions with their surfaces, was also proposed. Another mechanism based on disruption of the cell membrane and oxidative stress in the bacteria was proposed [22]. Based on earlier reports, nano-size ZnO particles have antibacterial activities by causing death or growth inhibition. Biological mechanisms of such activities have also been widely described and need not be rigorously described again here. Effects of different parameters on the disinfection process have also been thoroughly investigated in detail. In a recent review [23] the mode of action of nano-size ZnO particles has been surveyed. ZnO particles caused bacterial death and growth inhibition in the dark and under radiation. Bacterial inactivation by photo-catalytic processes has also been reported [24–26]. Antibacterial photo-catalytic activity of ZnO nano-particles with UV radiations and visible light has been recently investigated [27,28]. Hu et al., reported the anti-bacterial activity of ZnO/SiC nano-particles with no UV radiations [29].

Killing or inhibiting bacteria by ZnO particles, in the dark or under radiation, is assumed to yield a complex mixture of different organic compounds, which could themselves be hazardous. Despite the sizable number of reports on disinfection with ZnO nano-particles, to our knowledge, the issue of remaining organic matter after bacterial death in water has not been described in earlier literature [17,23,27,30–32]. TiO_2 particles can photo-degrade bacteria and their organic content, on the particle surfaces in the air [33], but in water the fate of the resulting organics has not been studied.

In a recent communication, we reported how dye sensitized ZnO nano-particles can be effectively used to kill *E. coli* and mineralize their organic content under visible radiations [34]. Despite the new findings, the tendency of the sensitizer to degrade under photo-electrochemical conditions, which needs continued addition of the dye to regenerate the sensitized catalyst, is a limitation for the sensitization technique. For wide scale practical purposes, it is necessary to use a robust catalyst system that functions under natural conditions with no need for continued regeneration.

It is assumed here that the ZnO nanoparticles will catalyze complete mineralization of bacteria and their organic contents in water under direct solar radiation. Such assumption will be tested in this work for the first time. ZnO nanoparticles were intentionally chosen here for a number of reasons. Firstly, ZnO is a non-hazardous material. Any Zn ion traces that may leach out of ZnO particles are non-hazardous to humans or to agriculture, as the World Health Organization (WHO) recommends using Zn as supplement [35–37]. Secondly the ZnO particles have band gaps (~3.2–3.3 eV) similar to other stable semiconducting materials such as TiO_2, with the advantage of having higher absorptivity toward the UV tail in the solar light [38–41]. Thirdly, the ZnO particles are easy to prepare in the nano-scale by simple methods from starting materials available in any laboratory. Moreover, ZnO exhibits soundly high photoconductive response which makes it useful in various applications [42]. It is widely described as a photo-catalyst for photo-degradation and other processes [43–46].

2. Experimental

2.1. Starting materials and solvents

Starting materials and other common lab chemicals, such as zinc chloride, barium chloride, nitric acid, sulfuric acid, ethanol, sodium hydroxide and hydrochloric acid were all purchased from either Sigma-Aldrich or Frutarom as analytical grade, and were used as received without further purifications.

2.2. Equipment

An AlaboMed Inc. spectrophotometer was used to quantitatively determine bacterial concentration using the turbidometric method. The suspensions were adjusted to the 0.5 M McFarland standard turbidity.

A Shimadzu UV-1601 spectrophotometer was used to measure the solid state electronic absorption (EA) spectra for the ZnO powders. The ZnO powder was cast onto the wall of a quartz cell.

A Perkin-Elmer LS50 luminescence spectrophotometer was used to measure the solid state photoluminescence (PL) emission spectra for ZnO powders in aqueous suspensions. The excitation wavelength was 325 nm.

Field emission scanning electron microscopy (FE-SEM/EDS) was measured on a Jeol Model JSM-6700F microscope. The service is available in the laboratories of ICMCB, University of Bordeaux, France.

X-ray diffraction (XRD) patterns were measured on a Philips XRD XPERT PRO diffractometer equipped with a Cu Kα radiation source (λ = 1.5418 Å). The service is available in the laboratories of ICMCB, University of Bordeaux, France.

Specific surface area measurement for the prepared solid ZnO was performed. The acetic acid adsorption method was used based on literature [35,47,48].

In the photo-catalytic experiments direct solar light was used as the irradiation source. Experiments were conducted under direct solar irradiation with average light intensity of 1000 lx (0.00015 W/cm^2). About 5% of the solar radiation is in the UV region 320–400 nm, while the major part (~95%) is in the visible and the IR regions [39,40].

Total organic content (TOC) was measured using a TELEDYNE TEKMAR TOC FUSION equipment, with a carbon detection limit range 2 ppb–10,000 ppm. The TOC method measures all organic carbon concentrations in the aqueous solution, including any living bacteria, bacterial surfaces and different organic compounds. Therefore, the TOC method gives an accurate measurement of all organic stuff remaining in the reaction mixture. Solid ZnO particles are not measured as they cannot be volatilized into CO_2 during oxidation, and remain in the oven.

Further analysis for the organic compounds, resulting from bacterial killing, was performed using gas chromatography/mass spectra (GC-MS). A Perkin-Elmer Clarus 500 GC/MS (2010) equipment, was used. The system is equipped with a SPME-GC/MS unit and an auto injector to directly analyze aqueous solutions. A capillary column (30 m in length and 0.25 mm in diameter) was used. Analysis conditions were as follows: starting with initial temperature 50 °C (for the first 10 min), the temperature was raised to 100 °C (ramp rate 2 °C/min) and kept for additional 20 min. The solid ZnO particles were filtered off from the aqueous mixture before injection into the GC/MS.

2.3. ZnO nanoparticles preparation

Nano-size ZnO particles were prepared as described earlier [49]. $ZnCl_2$ solution (0.45 M) was prepared by dissolving solid $ZnCl_2$ (15.23 g, 0.11 mol) in distilled water (200.00 mL). The solution was diluted to 250.00 mL. A solution of NaOH (0.90 M) was prepared by dissolving NaOH (9.00 g) in distilled water (200.00 mL) and diluting to 250.00 mL. The NaOH solution was then placed inside a 500 mL beaker and heated to ~55 °C. The $ZnCl_2$ solution was added drop-wise (within ~40 min) to the heated NaOH solution with vigorous magnetic stirring. The mixture was kept under these conditions for 2 h. The ZnO white precipitate was isolated, cleaned with deionized water and ethanol successively, and dried under air at ~60 °C. The prepared particles were characterized by UV–Visible absorption spectrophotometry, photoluminescence spectrometry, XRD and SEM as described below.

2.4. Bacterial solution preparations

2.4.1. The standard solutions

The 0.5 McFarland Standard solution was prepared from stock solutions of $BaCl_2{\cdot}2H_2O$ (1.175% w/v, 0.096 N) and H_2SO_4 (1%v/v, 0.36 N). Samples of H_2SO_4 (9.95 mL) and $BaCl_2$ (0.05 mL) solutions were mixed together. The optical density of the solution was in the range 0.08 – 0.10 when measured spectrophotometrically at λ_{max} 625 nm. This is equivalent to the optical density for bacterial formal concentration 1.5×10^8 CFU/mL.

A normal saline solution (0.9%) was prepared by dissolving NaCl (4.5 g) in distilled water (500 mL). NaOH and HCl solutions were prepared and used to adjust the pH of treated waters as desired. The NaOH (0.25 M) solution was prepared by dissolving solid NaOH (1.0 g) in 100 mL distilled water, and the HCl solution (0.25 M) was prepared by diluting concentrated HCl (33%) with distilled water (100 mL).

2.4.2. Bacterial strain, cultivation and quantification

Cultures of *E. coli* and *P. aeruginosa* were prepared similarly. The two bacteria were used as model test organisms in this work due to their wide abundance and their resistance to the bactericidal effects compared to other bacteria [50]. *E. coli* and *P. aeruginosa* of undesignated strains or serotype were isolated from clinical specimens and identified in the laboratories of Medical Laboratory Sciences, An-Najah N. University, according to standard diagnostic methods. All activities performed while handling bacteria were done under sterile conditions.

A nutrient broth was used for microorganism inoculum preparation. Both the nutrient agar and the nutrient broth were prepared according to the manufacturer (Oxoid Co.) instructions. The microorganism inoculums were prepared by inoculating a loop-full of the microorganism in 50 mL sterile nutrient broth and incubation at 37 °C for 7 h. The concentration of bacteria in the original broth was quantitatively determined to be 1.5×10^8 CFU/mL. This is equivalent to the 0.5 McFarland standard. To obtain a working cell suspension, an aliquot (167 µl) of the original concentration was mixed with 50 mL water in a beaker, with controlled pH (4.5, 7.04 and 9.0) to obtain $\sim5\times10^5$ CFU/mL concentration. The suitable pH value in this work was 7.05, and unless otherwise stated all studies conducted here involved pH 7.04. The nominal bacterial concentration was kept at $\sim5\times10^5$ CFU/mL and used in all disinfection experiments. The detection of viable *E. coli* and *P. aeruginosa* cells in the aqueous mixtures after each disinfection experiment was carried out by the plate count technique, as described below.

2.5. Disinfection experiments

Disinfection experiments were performed inside 100 mL sterile glass beakers. In a typical disinfection experiment, the diluted bacterial suspension (50 mL, with nominal concentration $\sim5\times10^5$ CFU/mL) was used with magnetic stirring. The beaker was dipped inside a thermostated water bath to maintain the temperature constant (~30 °C). The beaker was covered with aluminum foil before the start of each experiment to avoid contamination. ZnO nano-size powder (0.1 g) was added to the reaction mixture. The reaction mixture was then exposed to direct sun light (at measured radiation intensity of 1.5×10^{-4} W/cm^2, $\sim7.5\times10^{-6}$ W/cm^2 UV fraction) for 60 min with continued magnetic stirring. The number of viable bacteria in the treated mixture was quantified using the spread plate technique in which the sample was appropriately diluted with sterile saline solution (0.85% NaCl mass/mass) and transferred to agar plates. The plates of proper dilution that contained colonies from 30 to 300 were counted (Counts higher than 300 or lower than 30 CFU/plate were excluded based on standard microbiological practices) [51] and the numbers of bacteria in the original samples were calculated. The bacterial loss percentage was calculated, based on actual counts, by the formula:

$$(Bacterial\ \mathrm{Conc}_{init} - Bacterial\ \mathrm{Conc}_{fin})(100\%)/Bacterial\ \mathrm{Conc}_{init}$$

where $Conc_{init}$ is initial bacterial concentration counted at time zero, and Conc_{fin} is final concentration calculated for bacteria remaining after disinfection.

Different control experiments were conducted. One experiment was conducted in the dark with ZnO powder. Another experiment was conducted in the dark with no added ZnO powder. A photo-experiment was performed in the absence of any ZnO powder. An additional experiment was performed in the presence of ZnO powder under solar light while using a cut-off filter that eliminates any UV solar radiations with wavelength 400 nm or shorter. Another experiment was performed under UV radiation range (shorter than 400 nm) only in the absence of ZnO powder. A control experiment was conducted using a broth solution only (free of bacteria) with ZnO nano-particles under direct solar light. All disinfection and control experiments were repeated three times, and the results were calculated by averaging the measured values.

3. Results and discussion

3.1. ZnO characterization

ZnO powders have been heavily described in earlier reports. Despite that, literature [19,20] shows that ZnO particles with different characteristics may have various catalytic effects on bacteria. Therefore, characterizing the ZnO particles used here is needed. The prepared ZnO nano-particles were characterized by different methods. The solid state electronic absorption spectrum for the prepared ZnO particles is shown in (Fig. 1). The spectra show an absorption band at $\lambda_{max}=\sim380$ nm for the ZnO particles. The value resembles that reported earlier [52].

Photoluminescence spectrum measured for solid ZnO particles suspended in water, (Fig. 2), shows an emission band at 390 nm for the ZnO particles, in accordance with earlier reports [53]. The bands at 450 nm and longer wavelengths are due to the oxygen vacancies as described earlier [54,55].

The absorption and emission spectra indicate a band gap of ~3.2 eV, which is typical for ZnO particles. The spectra suggest that ZnO particles are in the nano-size scale [52].

The XRD pattern was measured for the prepared ZnO particles, and compared with earlier reports [56]. The particles mainly involved a wurtzite hexagonal phase. As shown in (Fig. 3), the ZnO particles exhibited six peaks with $2\Theta=31.88°$ for (100), 34.53° for (002), 36.36° for (101), 47.62° for (102) and 56.68° for (110) planes. The peaks are typical for the wurtzite structure.

The Scherrer equation was used to calculate the average particle size for the ZnO powder. Using the three diffraction peaks (102), (110) and (103), the average particle diameter was ~20 nm. The XRD pattern thus confirmed the nano-size of the prepared particles. The phase and

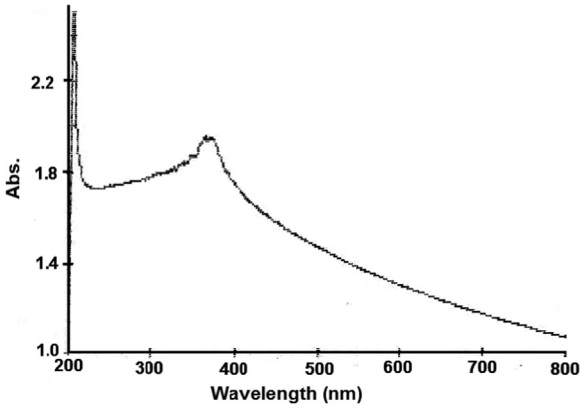


Fig. 1. Electronic absorption spectrum for ZnO particles suspended in water. Baseline correction was made vs. water.

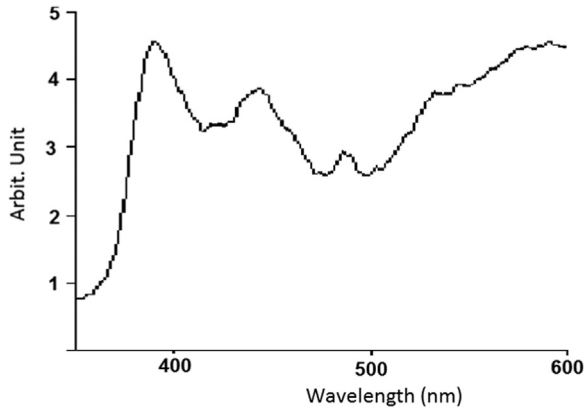


Fig. 2. Photoluminescence emission spectrum measured for solid ZnO particles suspended in water, using excitation wavelength 320 nm. Baseline correction was made vs. water.

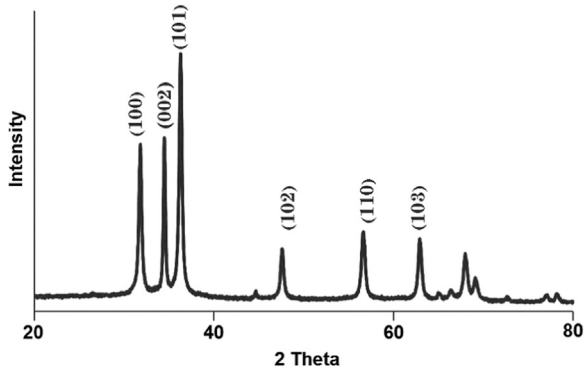


Fig. 3. XRD pattern measured for the prepared ZnO nano-particles.

the particle sizes of the prepared ZnO particles resembled those reported earlier [57,58].

SEM micrographs confirmed the nano-size nature of the prepared solid ZnO. Elongated rice-shaped ZnO agglomerates can be observed from (Fig. 4) with ~25 nm in diameter and 140 nm in length. The agglomerates involve smaller nano-size particles as confirmed by XRD above. The measured specific surface area for the prepared solid ZnO was ~45 m/cm^2.

3.2. Disinfection study

Water disinfection study was performed using the two types of bacteria mentioned above, *E. coli* and *P. aeruginosa*. Under direct solar radiation, ZnO nano-particles readily killed *E. coli* and *P. aeruginosa* and

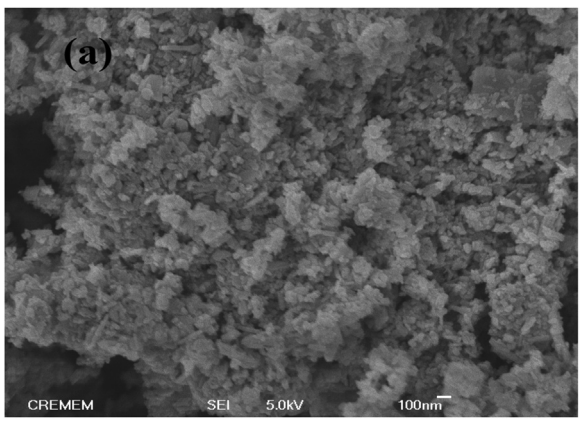


Fig. 4. SEM micrograph measured for the prepared ZnO particles.

completely mineralized organic compounds that resulted after their killing. The disinfection results are discussed here.

3.2.1. *E. coli bacteria*

ZnO nanoparticles showed anti-bacterial activity against *E. coli*, as summarized in (Table 1). The Table shows values of bacterial loss%, turnover frequency (TF, *bacterial CFU loss/Zn atom per h*) and quantum yield (QY, *bacterial CFU loss per UV photon*), in the dark and under solar irradiation. Different control experiments were performed. Entry (4) shows that in the absence of ZnO, no anti-bacterial activity was observed in the dark, as the *E. coli* concentrations remained constant within the experimental time (60 min). In the dark, the bacteria concentration was lowered by only ~20% in the presence of the ZnO particles. When ZnO particles were used under solar irradiation, the bacteria totally disappeared, (entry 1). Entry (2) shows that in the absence of ZnO particles the *E. coli* bacteria were not affected by radiation.

The anti-bacterial activity of ZnO nano-particles in the dark has been discussed above, and the *E. coli* ~20% loss, as shown in entry (3) of (Table 1), is consistent with that. Much higher activity occurred when direct solar radiation was used, with almost total disappearance of the bacteria, (entry 1).

Solar radiations involve a wide range of wavelengths, including mostly IR and visible, in addition to about 5% UV radiations [39,40]. The question now is which solar radiation range affected bacteria, in the presence and in the absence of ZnO particles? Since the reaction temperature was thermostated throughout photo-experiments, the IR effect should be ruled out, as IR radiations have only thermal rather than photochemical effects. The photo-reactions should then be due to either the visible or the UV radiations. Control experiments using visible light and longer waves only were performed for this purpose, using a cut-off filter that eliminates wavelengths 400 nm and shorter. In case of ZnO nano-particles, only 20(± 3)% bacteria disappeared, as shown in entry (5). The result resembles that of entry (3) (dark experiment with ZnO). Using the cut-off filter in the absence of any ZnO showed no loss in bacteria (entry 6), which resembles entry (4) in the Table.

The results clearly indicate that ZnO particles affected bacteria in the dark and to a larger extent under direct solar radiation. Moreover, the visible light did not significantly affect the ZnO activity on the bacteria.

The results indicate that ZnO has two modes of actions, in the dark and under UV radiation. In the dark, ZnO showed antibacterial activity that is limited to its effect on bacterial killing and growth inhibition. As discussed above, earlier reports manifested bacterial surface rupture and flow out of organic compounds by ZnO nano-particles. Therefore, the reaction mixture is assumed to contain all remaining living bacteria, ruptured bacteria, broth materials and other resulting organic materials. This has been confirmed here by studying the TOC, as summarized in (Table 2). The GC-MS analysis further confirmed the presence of a mixture of organic compounds after disinfection experiments. The TOC shows total organic content, in the reaction mixture, including living bacteria, dead (ruptured) bacteria, remaining broth and organic compounds resulting from bacterial death. In the dark, the TOC values resembled the nominal value, entries (2) and (3) of (Table 2). The results of (Tables 1 and 2) combined together indicate that ZnO particles kill the bacterial in the dark with no significant loss of the TOC after that. Entry (1) of (Table 2) shows also that exposure to light with no catalyst did not affect the TOC of the reaction mixture, which confirms entry (2) in (Table 1). Exposure of the bacterial mixture to solar light in the presence of ZnO particles, (Table 2) entry (4), shows totally different behavior. About 80(± 3)% loss of TOC occurred in this case, which confirms photo-mineralization of organic matter. In (Table 1), entry (1) showed complete killing of bacteria under light in the presence of ZnO particles. The TOC result here indicates that most organic matter resulting from such killing disappeared as mineral

Table 1
E. coli disinfection results under different conditions. All experiments were performed using 5×10^5 CFU/mL formal bacterial concentration for 60 min. The nominal (actually counted concentration) was 4.7×10^4 CFU/mL.

Entry Number	Experimental conditions	*E. coli* concentration after treatment (CFU/mL)*	Approximate *E. coli* loss (± 3%)[a]	TF	QY
1	Light/ZnO	~0	~100	3.2×10^{-15}	4.7×10^{-14}
2	Light/no ZnO	$\sim4.7\times10^4$	~0	0	
3	Dark/ZnO	$\sim3.7\times10^4$	~20	6.4×10^{-16}	9.4×10^{-15}
4	Dark/no ZnO	$\sim4.7\times10^4$	~0	0	
5	Light/ZnO (with cut-off filter)	$\sim3.7\times10^4$	~20	6.4×10^{-16}	9.4×10^{-15}
6	Light/no ZnO (with cut-off filter)	$\sim4.7\times10^4$	~0	0	

[a] Based on actual bacterial counting.

Table 2
Values of TOC measured for disinfection experiments under different conditions. Initial value of TOC at zero time was 32 ± 3 ppm.

Entry number	Experimental conditions	Value of TOC (ppm)
1	*E. coli*/Solar light/no ZnO	30 ± 3
2	*E. coli*/Dark/no ZnO	30 ± 3
3	*E. coli*/Dark/ZnO	30 ± 3
4	*E. coli*/Solar light/ZnO	7 ± 3
5	*Broth/Solar light/ZnO*	~0
6	*Broth/Dark/no ZnO*	16 ± 1

compounds. The remaining 7 ppm TOC (~20% of nominal bacteria) is due to the remaining bacterial cell walls that have not been mineralized. It is reported that the cell walls and the membranes of *E. coli* make up to 15–30% of the total bacteria [40,59,60]. Gram negative bacteria, like *E. coli*, have thin peptidoglycan cell walls which are made up of polysaccharide backbones [40]. In the dark, cell rupture occurred. This caused leaching out of internal organic materials to the reaction mixture [23]. In photo-catalytic mineralization, all resulting organics were further degraded into mineral materials, leaving only ruptured walls.

In (Table 2), entries (1–3), the TOC values were similar. This means that the total organic content in the disinfection mixture (which is the sum of living bacteria, dead bacteria and organic matters that leach out in addition to the remaining broth) remained unchanged in the absence of photo-catalytic activity of ZnO. Therefore, the antibacterial activity involved no mineralization. In entry (4), photo-mineralization occurred for the killed bacteria content and for the remaining broth itself. The control experiments (entries 5 and 6) indicate that the broth itself was completely mineralized with the ZnO photo-catalyst particles. Together, entries (4) and (5) confirm complete mineralization of all organic matter with the exception of the bacterial cell wall fragments. TOC results were confirmed by GC-MS analysis, as the organic content after photo-mineralization experiment was much lower than in other non-mineralization experiments.

3.2.2. *P. aeruginosa*

The *P. aeruginosa* followed similar behavior to that of *E. coli* in disinfection experiments. (Table 3) summarizes the disinfection results for the *P. aeruginosa* bacteria. Entry (3) shows that, in the dark, only ~25% of the bacteria were killed by the ZnO particles. This is consistent with the discussions presented above. In the absence of the ZnO particles no loss of bacteria was observed in the dark or under solar light, (entries 2 and 4). Under direct solar light, the ZnO particles caused nearly complete loss of the bacteria (entry 1). When a cut-off filter was used (entry 5) no bacterial loss occurred under direct solar light in the absence of ZnO. Collectively (Table 3) results indicate that ZnO may kill the bacteria in the dark and to a larger extent under direct solar light. The photo-catalytic activity of the ZnO particle occurred by the UV tail of solar light only, as evident from entries (1) and (6). Entry (6) shows that with a cut-off filter, the visible light does not affect the antibacterial activity of the ZnO particles. Entries (3) and (6) show that the ZnO particles cause partial loss of bacteria in the absence of UV light. Collectively, the results are in congruence with the *E. coli* results discussed in (Table 1) above.

Once killed, the *P. aeruginosa* bacteria are expected to leach out their organic content into the solution, as discussed in case of *E. coli*. In order to study the effect of the ZnO particles on the organic matter that resulted from the death of *P. aeruginosa*, the values of TOC remaining after the disinfection experiments were measured. (Table 4) summarizes the TOC values for reaction mixtures remaining after disinfection.

The Table shows that in the dark, the ZnO did not affect the value of the TOC of the bacterial suspension, entry (2). Therefore in the dark the ZnO particles killed the bacteria with no photo-catalytic mineralization of the organic matter. Entry (1) shows that only 8 ± 3 ppm (~25% of original organic materials) remained. This remaining TOC value is due to the bacterial surface fragments that resist complete mineralization, as discussed above. All other organic contents, including the broth itself, were completely photo-degraded with direct solar light, as evident from entries (1, 4 and 5). Entry (3) confirms this conclusion, as in the absence of UV the ZnO particles do not exhibit photo-catalytic mineralization due to their wide band gap of ~3.2 eV. In case of cut-off filter the ZnO particles may just kill the bacteria with no mineralization, and consequently the value of TOC resembles that for the dark experiments.

Collectively, the results show that both *E. coli* and *P. aeruginosa* bacteria encountered anti-bacterial effect by the ZnO nano-particles. Under photo-catalytic conditions, with direct solar light having a UV tail, the organic matters that leached out by bacterial killing underwent

Table 3
P. aeruginosa disinfection results under different conditions. All experiments were performed for 60 min exposure time using 5×10^5 CFU/mL formal bacterial concentration (nominal actually counted) 22.2×10^4 CFU/mL).

Entry number	Experimental conditions	*P. aeruginosa* concentration after treatment (CFU/mL)*	*P. aeruginosa* loss (± %)[a]	*TF*	*QY*
1	ZnO/light	0	100	3.0×10^{-16}	2.2×10^{-13}
2	No catalyst/light	22.2×10^4	0		
3	ZnO/Dark	$\sim16.75\times10^4$	~25	7.5×10^{-17}	5.56×10^{-14}
4	No catalyst/dark	$\sim22.2\times10^4$	0		
5	No catalyst/Light (Cut-Off filter)	$\sim22.0\times10^4$	0		
6	ZnO/Light (Cut-off filter)	$\sim17.0\times10^4$	~25	7.5×10^{-17}	5.56×10^{-14}

[a] Based on actual bacterial counting.

Table 4
Values of TOC measured for *P. aeruginosa* disinfection experiments under different conditions. Nominal value of TOC at zero time was 33 ± 3 ppm.

Entry number	Experimental conditions	Value of TOC (ppm)
1	ZnO/light	8 ± 3
2	ZnO/dark	33 ± 3
3	ZnO/light-with cut-off filter	33 ± 3
4	*Broth/Solar light/ZnO*	~0
5	*Broth/Dark/no ZnO*	16 ± 1

complete mineralization. Therefore, using the photo-degradation technique described here has the added value of killing both bacteria and mineralizing the resulting organic materials.

3.3. Modes of action of ZnO particles

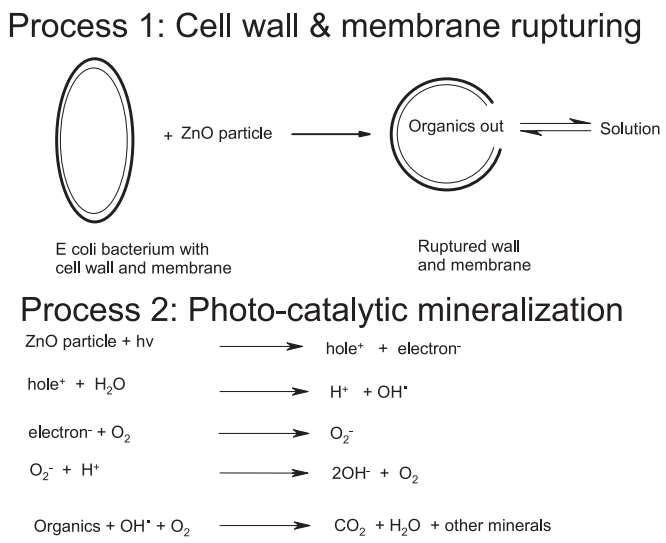
The results combined indicate two successive processes by which the ZnO particles function. In one way, the ZnO particles kill the bacteria, and in the other they photo-catalyze complete mineralization of the resulting organic matter.

Killing the bacteria in the dark by nano ZnO particles has been documented earlier by literature, as discussed above. The ZnO is believed to influence the bacterial surface causing release of the internal materials (including proteins, RNA and lipids) out of the cell to the solution. Such activity is enough to kill the bacteria. The reaction mixture is thus expected to involve the ZnO particles, released organic matter and other possible resulting compounds, in addition to the surface segments. In the dark, the resulting mixture involves different organic compounds. However, there is no reason for any of these organic compounds to degrade into mineral species such as CO_2. Therefore, killing bacteria in the dark should not affect the TOC in the reaction mixture.

In case of photo-catalytic process, things are different, as discussed above. The basic assumption was that organic materials released inside the reaction mixture should undergo complete photo-mineralization catalyzed by the ZnO particles. Such assumption has evidence from earlier reports, as complete photo-catalytic mineralization of other organic contaminants in aqueous solutions has been well reported by different nano-size materials [61–65]. The nano-size ZnO particles exhibit photo-catalytic activity in complete mineralization of different organic contaminants in water including phenol derivatives, drugs and industrial dyes [35,65,66]. Therefore, under light, the ZnO particles should photo-catalyze complete mineralization of organic matters that result from rupturing the bacterial cell walls in this work. Such a process is surely safer than bacterial killing only. The TOC results in (Tables 2 and 4) above confirm this assumption. In case of direct solar light, the TOC values show the absence of organic materials except the remains of the *E. coli* and *P. aeruginosa* cell walls themselves. In case of dark experiments, the TOC values showed no loss of TOC in the reaction mixture.

As discussed above, using ZnO particles under only visible light range, with UV tail cut-off, caused only partial death to the bacteria. This is expected, as the ZnO particles have band gap value of ~3.3 eV. This is equivalent to ~380 nm wavelength radiations. Therefore, the ZnO particles can be excited by 380 nm or shorter wavelengths only. When the cut-off filter was used no radiations with wavelength shorter than 400 nm were allowed to go through. Therefore, the ZnO particles could not be excited, and their activity is limited to killing the bacteria with no photo-catalytic mineralization.

The two modes of actions of the ZnO can be summarized by two successive processes as shown in Scheme 1. Each separate process is based on earlier reported mechanisms. As bacterial death mechanisms have been discussed in earlier reports [19,20,22,23,67], they need not be re-discussed here. By one way or another, the ZnO nano-particles rapture cell walls and cause death to the bacteria. This is summarized in process 1 of the scheme. Moreover, involvement of photo-catalysts in rupturing the bacterial cell walls may not be ruled out [23,68,69].



Scheme 1. A proposed mechanism showing how ZnO particles kill bacteria and photo-degrade their organic content.

The organic matters (mainly DNA, RNA and lipids) leach out of the dead cells to the reaction solution [23]. These organic matters are subject to complete photo-mineralization, as summarized in Process 2. The ZnO particles photo-catalyze the degradation of the resulting organic matter. The mineralization mechanism for such compounds should follow similar logic to that used for photo-mineralization of other different organic contaminants such as phenol derivatives, chlorinated hydrocarbons, dyes and others [70,71]. The reactions shown in Process 2 are those proposed earlier for mineralization of different organic contaminants in water [24,72]. The same mechanism explains the UV-driven mineralization of organic contents, once they leach out of the dead bacteria. The mechanism is mainly based on the ability a photon (with 390 nm or shorter wavelength) to excite a ZnO nano-particle. In this case an electron is excited from the valence band to the conduction band, yielding a hole in the valence band. A hole-electron pair is thus created. The hole and the electron are responsible for the creation of OH. radicals and O_2^- ions inside the reaction mixture. Once present the radical oxidizes the organic molecules until complete mineralization. The organic matters that result, by bacterial surface rupturing, are no exclusion, and thus undergo complete mineralization.

In addition to bacterial killing reported in earlier literature, this work confirms an additional mode for pristine ZnO nano-particles in water disinfection, where the resulting organic compounds are completely photo-mineralized. The described results are potentially useful for future low cost application where the ZnO particles can be suspended in natural water and can photo-catalyze complete disinfection by direct solar lights. To assess feasibility of the process at large application scales, work is underway here to study the effect of different natural conditions on the photo-degradation process. Investigating other gram positive types of bacteria, with different types of cell walls, is also underway. Mineralization of the cell walls will also be examined under different reaction conditions.

4. Conclusions

ZnO nano-particles killed *E. coli* and *P. aeruginosa* bacteria, partly in the dark and completely under direct solar radiation. In either case, the killed bacteria gave complex mixtures of organic compounds that leached into the reaction mixture. In the dark or under visible light only, the resulting organic matter did not degrade and remained in the mixture. Under direct solar radiation with UV tail, the bacteria were

killed and the resulting organic matters were completely mineralized, leaving the surface fragments only therein. In accordance with earlier studies where ZnO causes bacterial death, this work shows the added value of using ZnO as a photo-catalyst in mineralizing the resulting matter from bacteria death.

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