



Natural dye-sensitized ZnO nano-particles as photo-catalysts in complete degradation of *E. coli* bacteria and their organic content



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ABSTRACT

This communication describes for the first time how nano-size particles, sensitized with natural dye molecules of anthocyanin, can be used as catalysts in photo-degradation of gram negative *Escherichia coli* bacteria in water. The naked ZnO nano-particles degraded up to 83% of the bacteria under solar simulator light, while the dye-sensitized particles increased the bacterial loss by ~10%. Solar simulator light includes about 5% of UV tail (shorter than 400 nm) which means that both UV and visible light (longer than 400 nm) radiations could be involved. When a cut-off filter was used, the naked ZnO caused only 40% bacterial loss, in accordance with earlier literature that described killing of bacteria with ZnO particles both in the dark and under light. With the cut-off filter, the sensitized ZnO particles caused higher than 90% bacterial loss, which confirms sensitization of the ZnO particles to visible light. Moreover, the results show that the catalyzed photo-degradation process causes mineralization of the bacteria and their organic internal components which leach out by killing. The catalyst can be recovered and reused losing ~10% of its activity each time due to mineralization of the dye molecules. However, catalyst activity can be totally regained by re-sensitizing it with the anthocyanin dye. The effects of different experimental conditions, such as reaction temperature, pH, bacterial concentration and catalyst amount together with nutrient broth and saline media, will be discussed together with the role of the sensitizer.

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1. Introduction

Water contamination with different types of bacteria is a serious issue [1]. *Escherichia coli* are one class of widely spread gram-negative rod-shaped bacteria, with length of ~2 μm and width of ~0.5 μm. Depending on their type, *E. coli* may have hazardous effects such as diarrhea and gastroenteritis [2–4]. Water disinfection is a necessary practice which is performed by different methods such as chlorination [5,6], ozonation [7] and other costly

methods. Bacterial killing by nano-ZnO particles has recently been widely described as an alternative method [8–11].

Killing micro-organisms and inhibiting their growth by nano-particles has been reported [8–10]. ZnO nano powders have been used to kill *E. coli*, *S. aureus* and *Bacillus atrophaeus* [11,12]. ZnO particles caused bacterial death and growth inhibition in the dark and under radiation [13]. Both UV and solar simulator light have been described for bacterial death by photo-catalytic processes with ZnO nano-particles [14–16].

Gram negative bacteria, like *E. coli*, have thin peptidoglycan cell walls that are made up of polysaccharide backbones [17]. Literature suggested that the nano-particles kill the bacteria by different mechanisms of cell rupturing [13,18–21]. Different death

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mechanisms, including electrostatic interactions and membrane oxidation, have been described [13,18–21]. After cell rupturing, the internal organic matters (mainly DNA, RNA and lipids) leach out of the dead cells to the reaction solution [13]. Different studies, including more recent ones, were limited to the bacterial death and growth inhibition with no reference to what happens to the resulting organic compounds [13,14,16,22,23].

In a recent communication, we reported on what happens to gram negative *E. coli* bacteria when exposed to direct solar light in the presence of nan-size ZnO particles [24]. It has been observed that the UV radiations in the direct solar light completely degraded the killed bacteria, and the resulting organic content, into mineral substances. With the exception of traces of the damaged cell walls, no organic matter remained in the reaction mixture, which means complete mineralization of the killed bacteria and their organic content.

Utilization of ZnO (TiO₂) semiconductor surfaces to degrade organic compounds in water is well documented for phenol derivatives, drugs and industrial dyes [25–27]. Complete photo-mineralization of organic contaminants in aqueous solutions has been documented by different nano-size catalysts [25,28–31]. ZnO particles have relatively wide band gaps (~3.2 eV) which need radiations of ~387 nm or shorter wavelengths. Therefore, ZnO particles demand UV radiations to effectively catalyze photo-degradation processes. One way to increase photocatalytic efficiency of the ZnO particles is to increase their absorptivity in the UV region. A study showed that the ZrS₂/ZnO composite can be used in photodegradation of naphthol blue black contaminant under UV [32]. Another approach is to sensitize the wide band gap semiconductor to visible solar light as the UV radiations account for only 5% of the reaching solar radiations, *vide infra*.

In visible light or in the dark, the ZnO role would be limited to killing the bacteria by cell wall rupturing, while in the UV range the excited ZnO particles can undertake the photo-degradation catalytic role, and can cause complete mineralization for the killed bacteria and their organic matters. Despite that, the issue of using visible light range is still needed for many scientific and technical reasons. UV occurs in solar light only as a small proportion (~5%) [17,33]. Using the visible solar light would then be a virtue. For this purpose, the ZnO particles need to be sensitized to visible light like other earlier described systems [28,30]. Such technique has been widely described in Graetzel type solar cells and to a lesser extent in water purification from organic compounds. Therefore, dye-sensitized ZnO particles should be examined in complete mineralization of bacteria and their organic content. To our knowledge sensitizing ZnO particles, with natural dyes, in water disinfection by complete mineralization of bacteria and organic content has not been reported before.

The nature of the sensitizer should not be chosen arbitrarily. Metal-based dye systems, including ruthenium compounds and cadmium chalcogenide quantum dots, have been described as sensitizers in photodegradation studies. Subash et al. described how ZnO can be sensitized to solar light by adding Ag and CdS [34]. Based on cost analysis and environmental concerns, we do not recommend using metal based sensitizers, as they may possibly degrade into hazardous materials in water under photo-electro-chemical (PEC) conditions [28]. A safe alternative would then be using organic natural dye sensitizers for the ZnO particles, which are not hazardous by themselves [28,31]. Organic natural dyes may degrade out under PEC conditions, but the resulting compounds are expected to be mineral and non-hazardous. Therefore, it is strongly recommended to reconsider organic dye sensitizers for ZnO photo-catalytic processes.

The work aims at using anthocyanin (a well-known low cost and safe natural dye) sensitized ZnO nano-particles as photo-catalysts in disinfecting water from *E. coli* bacteria. The study will check if, unlike

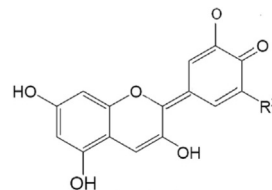
earlier systems, the sensitized catalyst can indeed function in the visible region or not. Secondly, the study will check if the killed bacteria (and their organic content) will again completely degrade into mineral compounds. To assess the future applicability of the described method in future perspectives, effects of different reaction conditions on the photo-degradation reaction progress will also be investigated. Recovery and regeneration of the catalyst system after reaction cessation will also be examined.

2. Experimental

2.1. General chemicals

Starting materials, organic solvents and common lab chemicals (such as sodium hydroxide, ethanol, barium chloride and mineral acids) were all purchased from either Aldrich–Sigma Co., or Frutarom Co., as analytical grade, and were used as received without further purifications. Commercial ZnO (99.9% purity) powder was purchased from Aldrich–Sigma Co. The ZnO was chosen here arbitrarily. Other materials provided by different suppliers could also be considered. Moreover, lab-prepared ZnO particles should be further examined in future activities.

The natural dye anthocyanin solution was obtained by extraction from dry dark-red *Karkade* (*Hibiscus*) flowers as described earlier [31]. The *Karkade* is a well-known safe flower commonly used in hot and cold drinks in many countries. Electronic absorption spectra [35,36] confirmed the presence of anthocyanin in the extract by showing the typical absorption band at 540 nm. Dry *Karkade* flowers (20.00 g) were finely ground in a mixer, soaked in 50.00 mL ethanol with continuous magnetic stirring for 30 min, and the extracted dye was then filtered. Few drops of concentrated HCl were added to the filtrate, to make the deep red solution more stable at pH < 1 [37]. The extracted dye (molecular structure shown below) was stored in a dark-color glass flask for further use.



Structure for anthocyanin dye extracted from *Karkade*

Based on the reported absorptivity in 1% HCl/methanol ($\epsilon = 34300 \text{ L mol}^{-1} \text{ cm}^{-1}$) [37] the anthocyanin calculated concentration in the solution was $5 \times 10^{-4} \text{ M}$.

2.2. General equipment

Suspension and solution electronic absorption spectra were measured on a Shimadzu UV-1601 Spectrophotometer. An AlaboMed Inc. Spectrophotometer was used to measure bacterial concentration using the turbidometric method. All suspensions were adjusted to the 0.5 M McFarland standard turbidity.

Photo-luminescence (emission) spectra were measured for solid suspensions on a Perkin-Elmer LS50 Spectrophotometer. Scanning electron microscopy (SEM) was measured on a Hitachi, Model S-4300, Field Emission Scanning Electron Microscope in *Korea Institute of Energy Research, Korea*. X-ray diffraction patterns were measured on a PANalytical X'Pert PRO X-ray diffractometer (XRD), with CuK α ($\lambda = 1.5418 \text{ \AA}$) at the *Pukyong National University, Korea*.

The solar simulator light was obtained from a 50 W solar simulator halogen spot lamp (LUXTEN). A Luxmeter (LX-102 light-meter) was used to measure the radiation intensity at the reaction

suspension. The measured intensity for the lamp radiation at the reaction surface was adjusted to resemble that for direct solar light at noon time in Nablus, Palestine, in the month of June (~ 70000 lx, 0.01025 W/cm²). The spectrum for the lamp also resembles that for direct solar light spectrum [38].

Specific surface area measurement for the solid ZnO was performed. The acetic acid adsorption method was followed here, as described earlier [26,39,40].

Total organic carbon (TOC) was measured on a TELEDYNE TEKMAR TOC FUSION system (carbon detection limit in the range 2 ppb–10,000 ppm, at JePharm Co. laboratories in Ramallah, Palestine. All organic carbon concentrations, including living and dead bacteria together with any resulting organics could be measured [24]. GC–MS spectra were measured on a Perkin-Elmer Clarus 500 GC/MS (2010) system equipped with SPME-GC/MS and auto-injector units. A 30 m long (0.25 mm in diameter) capillary column was used. The column oven starting temperature was set at 50 °C for 10 min, and raised to 100 °C (ramp rate 2 °C/min) for other 20 min.

2.3. ZnO/Dye preparation

Commercial ZnO powder (10.00 g) were mixed with ethanolic anthocyanin dye solution (50.0 mL, 5×10^{-4} M) and magnetically stirred for 30 min in a 100 mL beaker at room temperature. The mixture was left in the dark overnight. The solid was then filtered and thoroughly washed with ethanol and the dried in air away from light for further application. The dye uptake onto the solid surface (mmol dye/g solid) was calculated spectro-metrically using the absorptivity value for anthocyanin shown above. The number of moles remaining in solution was subtracted from the original number of moles used. The uptake value was $\sim 5 \times 10^{-4}$ mmol/g.

2.4. Bacterial suspension preparation and measurement

A 0.5 McFarland Standard solution was prepared from BaCl₂·2H₂O (1.175% w/v, 0.096 N) and H₂SO₄ (1.0% v/v, 0.36 N) stock solutions. Aliquots of H₂SO₄ (9.95 mL) and BaCl₂ (0.05 mL) solutions were mixed together. The solution optical density, measured at λ_{max} 625, was in the range 0.08–0.10, which is equivalent to the optical density of a 1.5×10^8 CFU/mL bacterial concentration.

The normal saline solution (0.9%) involved NaCl (4.5 g) dissolved in distilled water (500 mL). The nominal pH was adjusted as desired by using NaOH (0.25 M) and HCl (0.25 M) solutions.

The *E. coli* bacteria were used as model test here, because they are widely spread and have relatively high resistance [41]. *E. coli* of undesignated strains or serotype were isolated, based on standard methods, from clinical specimens and identified in the laboratories of the Medical Laboratory Sciences, ANU. Bacteria samples were all treated under sterile conditions.

The microorganism inoculum preparation was performed using a nutrient broth. The nutrient agar and the nutrient broth were prepared based on the manufacturer (Oxoid Co.) instructions. The inoculums were prepared by inoculating a loop-full of the bacteria in sterile nutrient broth (50 mL) and incubation for 7 h at 37 °C. The bacterial concentration in the original broth was measured

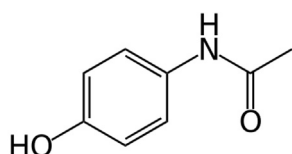
(1.5×10^8 CFU/mL) which is equivalent to the 0.5 McFarland standard concentration. The working cell suspension was prepared by diluting an aliquot (167 μ L) of the original broth with water (50 mL) in a beaker, with controlled pH (4.5, 7.04 and 9.0) to have $\sim 5 \times 10^5$ CFU/mL concentration. The suitable pH was 7.05, and unless otherwise stated this pH value was used throughout this work. The bacterial nominal concentration was kept at $\sim 5 \times 10^5$ CFU/mL and used in different disinfection experiments. The viable *E. coli* cells in aqueous mixtures, in disinfection experiments, were detected by the plate-count technique.

2.5. The photo-catalytic disinfection experiment

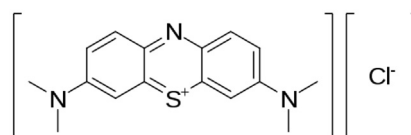
Photocatalytic disinfection was conducted under air inside 100 mL sterile glass beakers. In a typical experiment the procedure was as follows: Diluted bacterial suspensions (50 mL, with nominal concentration $\sim 5 \times 10^5$ CFU/mL) were used in disinfection experiments. The beaker was thermostated in a water bath at constant temperature (~ 30 °C). To avoid contamination, the beaker was covered with aluminum foil before the experiment. ZnO powder (0.1 g) was added to the beaker. The magnetically stirred reaction mixture was exposed to solar simulator light (at measured radiation intensity of 0.01025 W/cm²) for 60 min. The number of viable bacteria in the suspensions (before and after disinfection experiments) was measured using the spread plate technique. Samples (1.0 mL each) were taken from the reaction mixture. Each sample was diluted with sterile saline solution (9.0 mL, 0.85% NaCl mass/mass). Successive dilutions were performed based on standard methods. A sample (0.1 mL) of each dilution was uniformly spread on the plate and incubated at 37 °C for 24 h. Three plates were used for each dilution. The three plates with proper dilution that contained colonies from 30 to 300 were counted and the average number of bacteria was taken (Counts higher than 300 or lower than 30 CFU/plate were excluded based on standard protocols) [42]. The initial and final concentrations of bacteria were calculated. The bacterial loss percentage was calculated by the formula $(\text{Bacterial Conc}_{\text{init}} - \text{Bacterial Conc}_{\text{fin}})(100\%)/(\text{Bacterial Conc}_{\text{init}})$. Organic content in the reaction mixture was measured using the TOC and GC/MS methods described above.

Control experiments were conducted. Control experiments were performed in the absence of any catalyst in the dark and under radiation. Dark experiments were performed using both naked and sensitized ZnO particles. Anthocyanin dye was used alone with no ZnO in the dark and under radiation. Cut-off filters were used to eliminate radiations with wavelengths shorter than 400 nm in some experiments. All disinfection reactions, including control experiments, were repeated three times, and the results were calculated by averaging the measured values.

Other control experiments were performed on organic water contaminants with no added bacteria. The experiments were made to check ZnO/Dye ability to photo-degrade organic compounds that may result from bacterial death, as described in Section 1 above. Two organic compounds were examined here namely: paracetamol, a common drug compound that is reported as a water



Paracetamol Structure



Methylene Blue Structure

contaminant [43] and methylene blue (a widely studied model organic contaminant). In each case a 50.0 mL solution of the organic contaminant (10 ppm) was mixed with the ZnO/Dye (0.1 g) and exposed to visible light (wavelengths longer than 400 nm) using a cut-off filter at room temperature for 60 min.

3. Results and discussions

3.1. Characterization results

The ZnO/Dye catalyst system was characterized by a number of methods, as described earlier [24]. As shown in Figure (S1), the electronic absorption spectra confirmed the presence of anthocyanin onto the prepared catalyst. The spectra (Figs. S1 a&b) show an absorption band characteristic for the ZnO at ~ 387 nm (~ 3.2 eV), and another band at ~ 600 nm (2.1 eV) for the adsorbed anthocyanin. The band at 600 nm shows a shift from 550 nm characteristic for the ethanolic solution of anthocyanin shown in Figure (S1c). The shift is an indication for chemisorption of anthocyanin molecules onto the ZnO solid surface. Similar behaviors were observed for anthocyanin molecules anchored to other solid surfaces such as TiO₂ [31]. Literature [44] shows that anthocyanin has a band gap of 2.1 eV depending on the medium pH, *vide the mechanism section*.

The PL emission spectra measured for the naked and sensitized ZnO particles are shown Figure (S2). Due to the low concentration of the supported molecules their emission spectrum is weak to observe in Figure (S1a), whereas the PL spectrum for the in solution anthocyanin can be clearly observed in Figure (S1b). The sensitized ZnO particles showed about 3 fold enhancement in their PL emission intensity. This is due to enhanced surface by the sensitizer molecules which behave as passivating agents in this case. Surface modification has been reported to enhance semiconductor PL emission intensity by removing surface states [45].

Fig. 1 shows the measured XRD patterns for naked and anthocyanin sensitized ZnO powders. The peaks at $2\theta = 31.67^\circ$, 34.31° , 36.14° , 47.40° , 56.52° , 62.73° , 66.28° , 67.91° and 69.03° are due to (100), (002), (101), (102), (110), (103), (200), (112) and (201) in ZnO, respectively. The patterns confirm the polycrystalline wurtzite structure for ZnO in both cases [46]. The average particle size value for both powders, calculated by the Scherrer's formula, was ~ 60 nm. The resemblance between the two patterns is due to the molecular nature of the anthocyanin that is attached on the ZnO surface. The sensitizer should therefore not affect the crystal structure of the ZnO particles.

The SEM micrographs for both naked and sensitized ZnO particles are shown in Fig. 2a and b. The micrographs show elongated agglomerates with average width ~ 200 nm and average

length less than 500 nm. The agglomerates involve smaller nano-size particles of ~ 55 nm as measured by XRD patterns. Other details of ZnO solid particle characterization results were rigorously discussed earlier [24]. The specific surface area values, measured by acetic acid adsorption as described above, for the naked and anthocyanin sensitized ZnO solids were less than 20 m²/g. In congruence with XRD results, the SEM results indicate that the attached dye molecules did not affect the ZnO particles. Again this is due to the molecular nature of the attached anthocyanin.

3.2. Photo-catalytic disinfection

Efficiency of anthocyanin-sensitized ZnO particles in degrading *E. coli* bacteria under solar simulator light has been investigated. The results are discussed here.

3.2.1. Type of photo-catalyst

Fig. 3 shows bacterial loss percentage values when using different catalyst systems under solar simulator light and in the dark. In control experiments, the *E. coli* bacteria were not affected by the anthocyanin when used alone. In the dark, about 30% loss of bacteria occurred by naked and by sensitized ZnO particles due to death, with no mineralization. TOC confirmed these results, as the total organic content did not decrease, Table 1b and c). Both homogeneous and supported dye molecules showed no effect in the dark. Similar reports show that free anthocyanin solutions do not affect *E. coli* bacteria [47]. In the absence of any catalyst, $\sim 40\%$ bacterial loss was observed under solar simulator light with no TOC lowering, Table 1a.

When the ZnO particles were exposed to solar simulator radiations, up to 83% loss of bacteria was observed, Fig. 3. This is paralleled with about 65% lowering in TOC as shown in Table 1d. The sensitized ZnO catalyst showed higher activity with $\sim 94\%$ bacterial loss. The anthocyanin dye enhanced the efficiency of ZnO under solar simulated light by about 11%. Nearly complete mineralization of the killed bacteria was also witnessed by TOC measurements, Table 1e.

Fig. 4 shows photo-degradation results observed using visible light only (with a cut-off filter). In the visible light, the ZnO/Dye system caused bacterial loss of $\sim 92\%$. More than 85% lowering in TOC is observed in Table 1e. The naked ZnO killed only 40% of the bacteria under visible light with incomplete mineralization of organic compound mixture within the reaction duration. Table 1f shows $\sim 45\%$ lowering in TOC. Under simulated solar light (no cut-off filter) the naked ZnO and sensitized ZnO systems caused 83% and 92% bacterial loss, respectively, as described above in Fig. 3. Therefore, the sensitized ZnO catalyst functions effectively in the visible light and in the UV radiation tail, while the naked ZnO catalyst functions effectively in the UV tail only.

Collectively, the results indicate that the naked ZnO particles kill *E. coli* bacteria in the dark and under visible light, and completely mineralize the killed bacteria under UV radiations, in congruence with earlier report [24]. The sensitized ZnO particles kill the bacteria in the dark, while under visible and UV radiations they completely mineralize them. As the naked ZnO was studied earlier, attention is paid to sensitized ZnO particles here. In addition to TOC study, GC-MS results confirm *E. coli* photodegradation, Figure (S3). During reaction organic compounds appeared clearly (Fig. S3a). With time, the organic compounds disappeared, Figure (S3b). Figure (S3c) shows the occurrence of a complex mixture of organic compounds during the reaction.

Control experiments conducted on broth only, with no bacteria showed that the broth was completely mineralized, Table 1g. This means that the broth itself degrades during photo-degradation and does not affect the TOC results described in Table 1a–f.

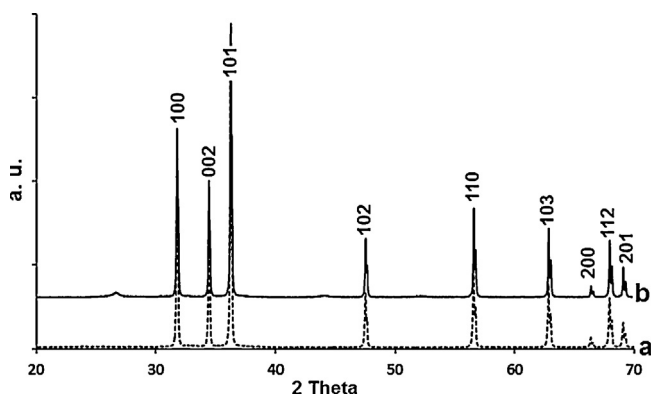


Fig. 1. XRD patterns for solid ZnO powders a) naked b) sensitized.

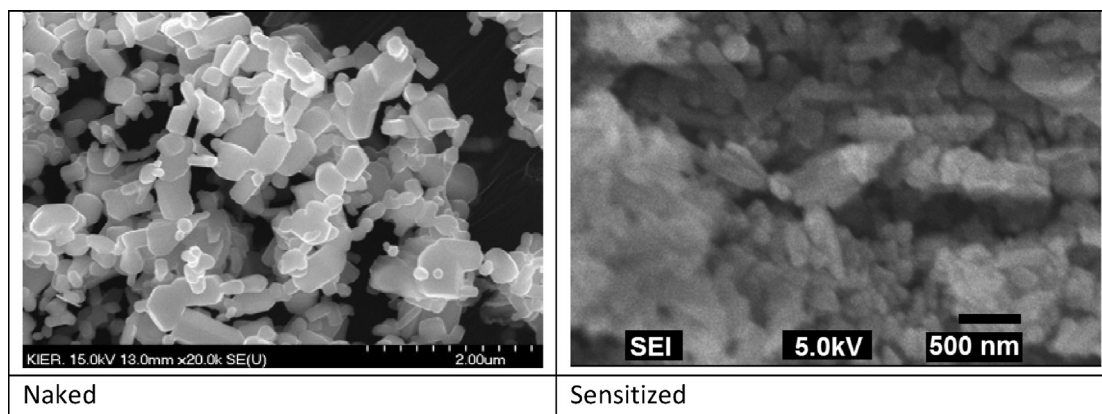


Fig. 2. SEM micrograph for solid ZnO powders (a) Naked, and (b) Sensitized.

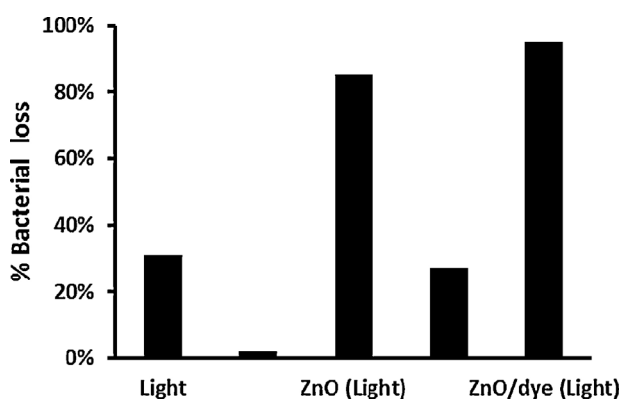


Fig. 3. Results of bacterial loss percent measured under different experimental conditions using different catalyst systems. All experiments were performed using *E. coli* suspensions (50.0 mL neutral suspensions, 3.00×10^5 cfu/mL) at room temperature for 90 min. Light experiments were performed under 0.0102 W/cm^2 . Catalyst loading was 0.100 g.

3.2.2. Factors affecting sensitized ZnO catalyzed disinfection

3.2.2.1. Effect of temperature. The anthocyanin sensitized ZnO particles were used to catalyze solar simulator light-induced degradation of *E. coli* at different temperatures. The *E. coli* themselves are affected by temperature. The optimal temperature range for *E. coli* living is $20\text{--}37^\circ\text{C}$, and this range was used here to neutralize the effect of temperature on the bacteria themselves. Fig. 5 summarizes the effect of temperature on bacterial percentage loss. The catalyst activity slightly increased with increasing temperature. This is consistent with earlier studies on organic contaminant [25–27]. In photo-catalytic degradation of organic contaminants in water, the source of excitation is either the UV or the visible radiations, with energy range of $3.2\text{--}1.6 \text{ eV}$. The thermal energy is far less than these values (less than 1.5 V) which

may not excite the catalyst particles. Therefore, the temperature effect on the reaction rate is restricted to diffusion control only.

3.2.2.2. Effect of pH. Effect of pH on anthocyanin-sensitized ZnO catalyst efficiency was studied in a pH range that is suitable for *E. coli*, and causes no death or growth inhibition. Three pH values (5.0, 7.5 and 8.7) were used as nominal values. In each case, the pH value for the reaction mixture changed to be in the range 7.3–7.6. Within the nominal pH values used, there was no significant effect in catalyst efficiency. The basic medium showed slightly higher efficiency than the acidic one. Fig. 6 summarizes these results.

3.2.2.3. Effect of catalyst concentration. The effect of added amount of sensitized-ZnO catalyst on the *E. coli* percentage loss was studied. Increasing the catalyst amount increased the *E. coli* loss percentage, up to a limit. Using more than 0.1 g catalyst loading caused lowering in *E. coli* loss percentage. Fig. 7 summarizes these findings. The results show that, under the experimental conditions used, the optimum catalyst loading was 0.1 g catalyst in 50 mL bacterial suspension.

The results of Fig. 7 are summarized in Table 2. The Table shows values of efficiency for the dye-sensitized ZnO catalyst, expressed in terms of turnover frequency (TF = Degraded bacteria per (Zn atom) per min) and quantum yield (QY = Degraded bacteria per incident photon). The results confirm the 0.1 g catalyst amount to be the optimum value in the series.

In principle, the reaction rate should increase with increasing catalyst loading, because with higher catalyst concentration more catalytic sites are accessible to the reactants. In nano-particle photo-catalytic studies, it is common to see the reaction slowing down with higher catalytic amounts [26,28,30]. One possible reason is due to the tendency of the catalyst particles, present at the suspension surface, to screen oncoming light away from other particles in the bulk of the suspension. Therefore, an optimal amount of catalyst loading is needed in such systems.

Table 1

TOC values measured for mixtures of different photo-degradation experiments of bacteria under different conditions.

Entry Number	Experimental conditions	Remaining TOC (ppm)
A	<i>E. coli</i> /Solar simulator light/no catalyst	35 (± 3)
B	<i>E. coli</i> /Dark/no catalyst	37 (± 3)
C	<i>E. coli</i> /Dark/ZnO/Anth.	35 (± 3)
D	<i>E. coli</i> /Solar simulator light/cut-off filter/ZnO/Anth.	10 (± 3)
E	<i>E. coli</i> /Solar simulator light/ZnO	5 (± 3)
F	<i>E. coli</i> /Solar light/cut-off filter/ZnO	20 (± 3)
G	Broth/Solar simulator light/ZnO/Anth	~ 0

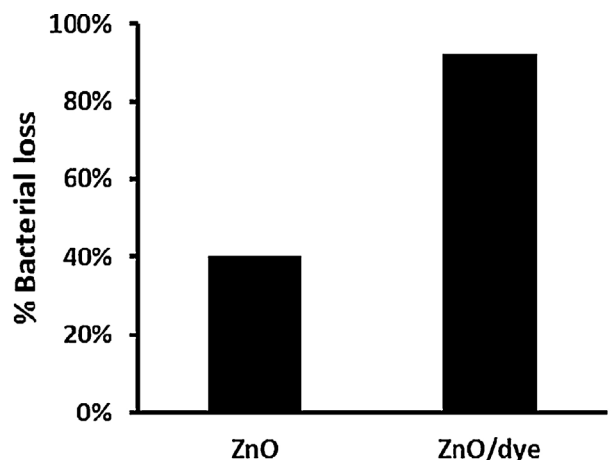


Fig. 4. Loss of *E. coli* under visible light by naked ZnO and sensitized ZnO systems. Experiments were conducted using 0.0102 W/cm^2 solar simulator light with a cut-off filter. Neutral aqueous suspensions (50.00 mL , $3.00 \times 10^5 \text{ cfu/mL}$ *E. coli* bacteria) with 0.100 g catalyst, at room temperature for 90 min , were used.

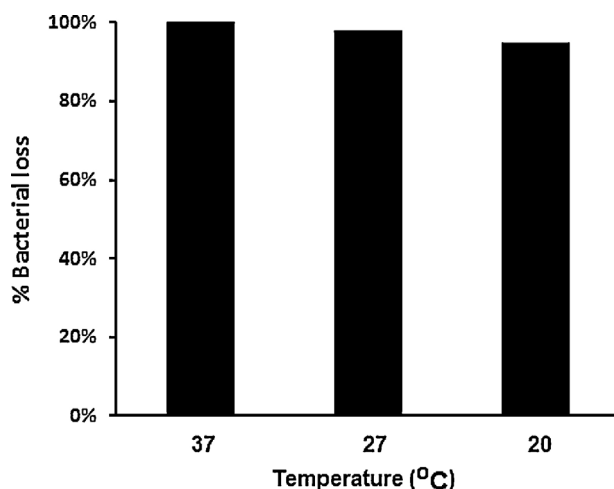


Fig. 5. Temperature effect on bacterial loss. All experiments were conducted using sensitized ZnO catalyst (0.100 g) in 50.00 mL neutral suspensions of *E. coli* ($4.70 \times 10^5 \text{ cfu/mL}$) for 90 min under solar simulator light (0.0102 W/cm^2).

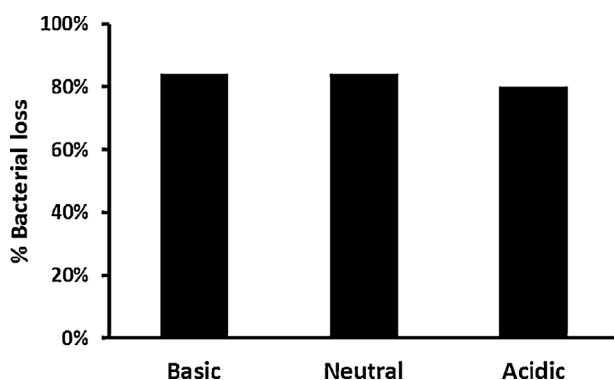


Fig. 6. Effect of nominal pH on bacterial loss. All experiments were conducted using sensitized ZnO catalyst (0.100 g) in 50.00 mL suspension of *E. coli* ($5.00 \times 10^5 \text{ cfu/mL}$) under solar simulator light (0.0102 W/cm^2) at room temperature for 90 min .

3.2.2.4. Effect of *E. coli* concentration. Fig. 8 shows how the nominal *E. coli* concentration affects their loss percentage. The Figure results are summarized in Table 3. The Table shows that the

bacteria loss percentage goes higher as the nominal bacterial concentration was increased up to a limit. At concentrations higher than $\sim 5.0 \times 10^5 \text{ cfu}$, the percentage loss decreases. This should not be confusing, and the absolute number of the lost bacteria still increased with higher nominal bacterial concentration. The values of TF and QY continued to increase with higher nominal bacterial concentrations, as shown in Table 3. The results are understandable, as keeping all other variables constant, higher number of bacteria gives higher probability for bacteria to react. Table 3 shows the practical potential of the process as more bacteria are killed with higher concentration. Using the process in heavily contaminated waters should thus be considered for the future.

3.2.2.5. Effect of medium nature. Using added broth in the photo-catalytic experiments lowered the bacterial loss. In experiments where the original broth solution was diluted with 50 mL of pure water, bacterial loss higher than 94% was observed in 90 min , which is consistent with abovementioned results. When the original bacterial broth solution was diluted with 50 mL broth solution, 75% bacterial loss was observed, Fig. 9. The effect of the broth is understandable as it degrades readily along with the bacteria [24]. Therefore, degradation of bacteria and the broth need longer time. Moreover, the broth itself may cause screening of light from the catalytic active sites. In either case, with or without broth, the described process is potentially valuable for disinfecting waters with and without additional organic materials.

Using NaCl (normal saline) solution in bacterial suspension preparation showed only little effect on the percentage loss, as shown in Fig. 10. In photo-degradation experiments conducted with no added NaCl, by diluting original bacterial broth solution with 50 mL of distilled water, more than 90% loss was observed in 90 min . When NaCl was added to the reaction mixture, by using 50 mL of normal saline instead of distilled water, only $\sim 87\%$ bacterial loss was observed. This is not unexpected, because normal saline is known to protect bacteria by the osmotic pressures phenomenon. The fact that the technique described here is effective in both saline and non-saline media shows its added value in practical processes.

3.2.2.6. Effect of catalyst recovery. The sensitized ZnO particles were recovered from a photo-catalytic experiment and reused for fresh bacterial batches. The recovery was performed by a facile method, as the reaction mixture was taken and autoclaved (at 120°C under 1.5 atm) for enough time (15 min). The catalyst particles were then isolated by suction filtration and used in a fresh reaction batch. Second recovery was also performed on the same sensitized ZnO catalyst particles. In each case, up to 10% lowering in bacterial percentage loss was observed, Fig. 11. The dye molecules themselves may partly degrade under the photo-reaction conditions. This resembles other metal based dyes, which degrade under contaminant photo-degradation processes, as discussed in Section 1 above. Catalyst efficiency loss on catalyst recovery and reuse is common [48,49].

The catalyst de-activation on re-use is not due to poisoning, but due to sensitizing dye molecule degradation, as the sensitized ZnO powder color changed after its recovery. This was further confirmed by re-activating the recovered particles by simply re-dyeing them. The recovered catalyst particles were treated with anthocyanin solution as described earlier in Section 2. The re-sensitized particles were then re-used in fresh photo-degradation experiments. Fig. 12 shows that the re-sensitized ZnO particles retained their original efficiency just like fresh sensitized catalyst systems. The ability to recover and re-sensitize ZnO particles is an added value for the method described here, in two aspects. Firstly, the degraded dye molecules showed no traces of organic

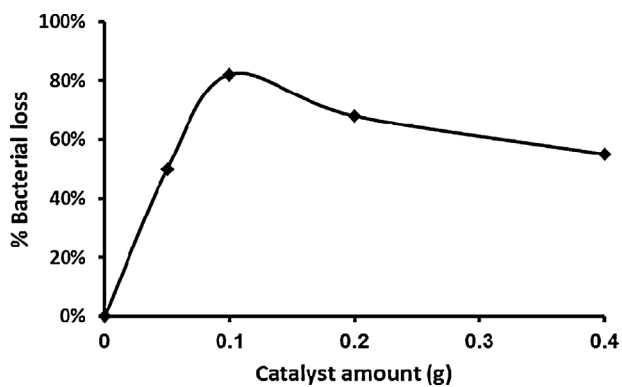


Fig. 7. Effect of sensitized ZnO catalyst concentration on bacteria loss. All reactions were conducted under solar simulator (0.0102 W/cm^2), in neutral 50.00 mL suspension of *E. coli* ($4.00 \times 10^5 \text{ cfu/mL}$), for 90 min at room temperature.

Table 2
Effect of catalyst amount on its efficiency, expressed in terms of bacterial loss percentage, TF and QY.

Catalyst mass (g)	Bacterial Loss%	TF (min^{-1})	QY
0.050	50%	1.415×10^{-16}	42.846×10^{-16}
0.100	82%	2.303×10^{-16}	69.713×10^{-16}
0.200	67%	1.899×10^{-16}	57.507×10^{-16}
0.400	55%	1.554×10^{-16}	47.051×10^{-16}

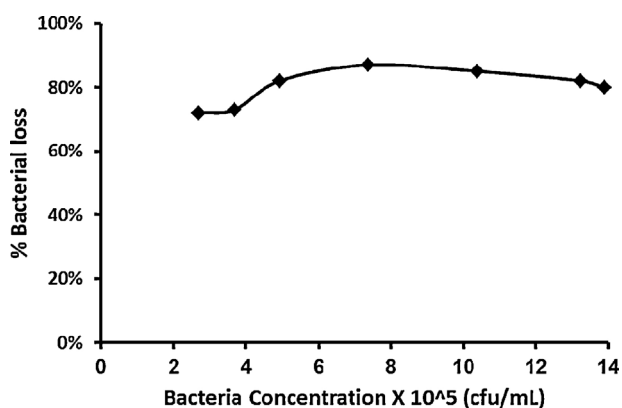


Fig. 8. Bacteria concentration effect on the bacterial loss. All experiments were conducted using sensitized ZnO catalyst (0.100 g) for 90 min under solar simulator light (0.0102 W/cm^2) at room temperature, in neutral 50.00 mL suspensions. Bacteria concentrations were 2.67×10^5 , 3.70×10^5 , 4.93×10^5 , 7.37×10^5 , 10.40×10^5 , 13.25×10^5 and $13.90 \times 10^5 \text{ cfu/mL}$.

compounds, which proves the safe use of anthocyanin dye, (Section 1 above). Secondly, the catalyst particles can be re-activated in future large scale processes by a simple re-dyeing method. Such a strategy is being investigated here at larger (Liter) scale.

Table 3
Effect of bacterial concentration on bacterial loss.

Bacteria concentration (cfu/mL)	Bacteria Loss%	TF (min^{-1})	QY
2.67×10^5	72%	1.445×10^{-16}	43.755×10^{-16}
3.7×10^5	73%	2.02×10^{-16}	61.144×10^{-16}
4.93×10^5	82%	3.048×10^{-16}	92.283×10^{-16}
7.37×10^5	87%	4.794×10^{-16}	145.072×10^{-16}
10.4×10^5	85%	6.644×10^{-16}	200.933×10^{-16}
13.25×10^5	82%	8.168×10^{-16}	246.961×10^{-16}
13.9×10^5	80%	8.316×10^{-16}	252.7270×10^{-16}

3.3. Catalyst sensitization mechanism

The mechanism of UV driven complete mineralization of bacteria and organic content, by naked ZnO particles, was discussed earlier [24]. A two stage process was proposed, namely rupturing the bacterial surface wall and leaching out of the organic content into solution, a process that is well documented in literature with various mechanisms [13,18–21]. This was followed by mineralization of the resulting organic matters. The photo-degradation was restricted to the UV radiations which readily excited the naked ZnO nano-particles themselves.

In this work, a sensitizer was used, and a cut-off filter was used to eliminate UV. Therefore, the visible light is involved in the excitation process. Again the photo-degradation process is expected to follow death and cell rupturing of the bacteria, where organic components flow out into the aqueous suspension. In the photo-degradation process, the visible light photons excite the dye molecules not the ZnO particles themselves. One photon initiates electron-hole production, where the electron goes to the LUMO level of the dye and the hole remains in the HOMO levels, as shown in Scheme 1. Similar formalisms have been proposed earlier [25,28–30,50] for sensitized catalysts in photo-degradations of organic contaminants. Subash et al. showed how ZnO particles can be sensitized to solar light when co-doped with Zr and Ag, and the Zr-Ag-ZnO composite showed higher catalytic activity in the solar driven mineralization of organic contaminants [51]. In another more recent report [34] the CdS sensitized Ag-ZnO composite readily mineralized stable organic contaminants in water. Therefore the ability of sensitized ZnO particles to catalyze complete mineralization of organic molecules in water with visible solar light is possible. The report showed that two concurrent catalytic processes may occur, one by the sensitization (visible light absorbed by the CdS) and another with no sensitization (UV light absorbed by TiO_2). Photo-degradation of organic compounds resulting from bacterial death is thus possible by sensitized ZnO particles here.

One may argue the ability of the visible light to degrade organic molecules in water. In fact the oxidation of organic compounds, by oxygen, is a thermodynamically feasible and spontaneous process. It should occur indifferently in water or in the gaseous phase. However, the presence of water slows down the process, and higher activation energies are thus needed for oxidation in water. The role of the photo-catalyst here is to kinetically speed up the oxidation process, which is thermodynamically feasible in nature. Therefore, the ability of the anthocyanin sensitized ZnO in complete mineralization of the bacteria and their organic content should not be ruled out. The dye molecules are responsible for sensitizing the catalyst to visible solar light, as reported earlier for sensitized TiO_2 particles [52].

Scheme 1 shows two concurrent mineralization processes, one through sensitization and the other with UV. However, as UV is excluded by the cut-off filter, the former process is responsible for mineralization. This has been confirmed here. As described in Section 2 above, special control experiments were performed to confirm the ability of the catalyst systems to photo-degrade two

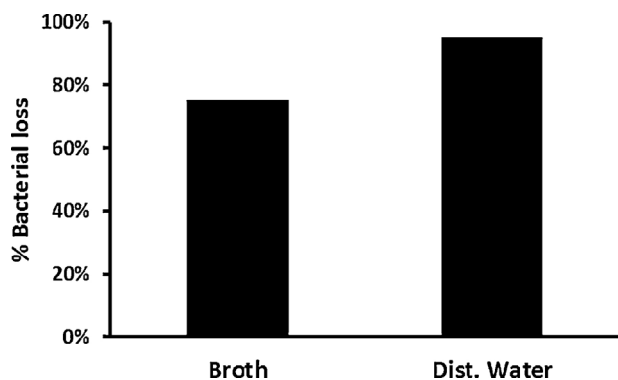


Fig. 9. Effect of broth on bacterial loss. All experiments were conducted using ZnO/anthocyanin (0.100 g) in neutral 50 mL suspensions of *E. coli* ($\sim 3 \times 10^5$ cfu/mL) under solar simulator (0.0102 W/cm^2) at room temperature for 90 min.

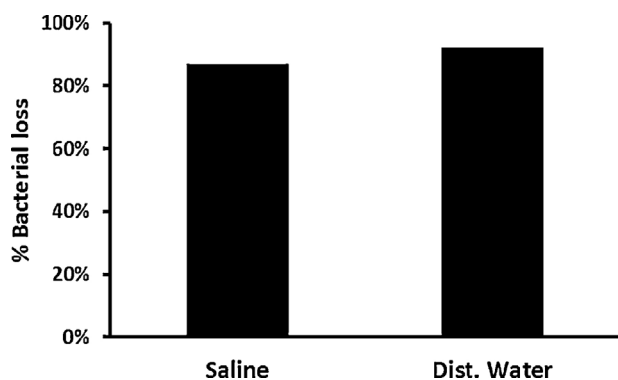


Fig. 10. Effect of normal saline the bacterial loss. All experiments were conducted using sensitized ZnO (0.100 g) in neutral suspensions (50 mL) of *E. coli* ($\sim 5 \times 10^5$ cfu/mL) under solar simulator light (0.0102 W/cm^2) at room temperature for 90 min.

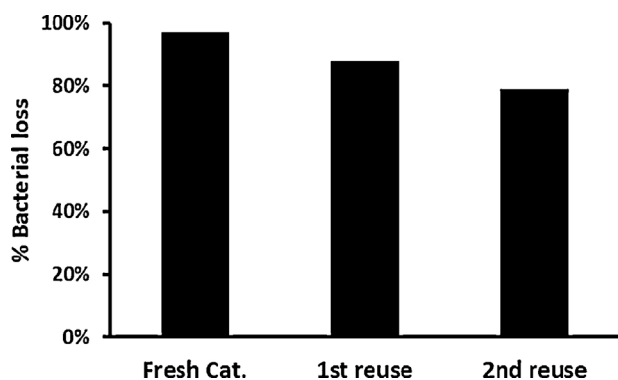


Fig. 11. Bacterial loss on catalyst recovery and reuse. Experiments were conducted using fresh (0.10 g), first recovered and second recovered sensitized ZnO catalyst. In each experiment, a fresh neutral (50.00 mL) suspension of *E. coli* (5.00×10^5 cfu/mL) was used under solar simulator light (0.0102 W/cm^2) at room temperature for 90 min.

organic compounds by the visible solar light. The anthocyanin sensitized ZnO catalyst caused up to 70% degradation of the paracetamol, and up to 80% methylene blue in water. In addition to the remaining contaminant molecules, no other organic contaminants were detected in the reaction mixtures. This was evidenced by spectral analysis of the reaction mixtures, which showed no other developing new bands due to formation of new organic products, as shown in Fig. 13. The results confirm the ability of the anthocyanin sensitized ZnO particles to mineralize organic materials in water with visible solar light, as described in the

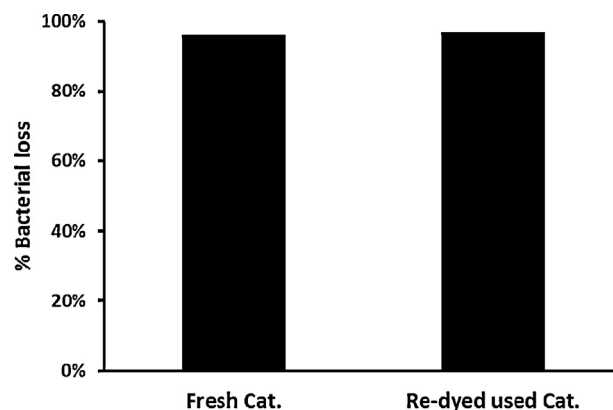
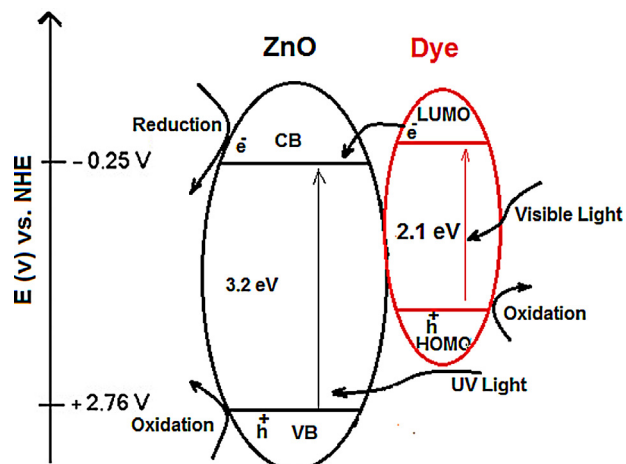


Fig. 12. Effect of re-sensitizing the recovered ZnO catalyst on its catalytic efficiency. Experiments were conducted using 0.1 g sensitized ZnO catalysts in neutral suspension (50.00 mL) of *E. coli* (10.0×10^5 cfu/mL) under solar simulator radiation (0.0102 W/cm^2) at room temperature for 90 min.



Scheme 1. Schematic sensitization of ZnO particles with anthocyanin molecules for visible light.

Scheme. Complete mineralization of reacted organic compounds under PEC conditions has been documented by earlier [34,51].

Different mechanisms have been successfully proposed for photo-degradation of organic compounds in water [16,52,53], by sensitized or naked semiconductor particles. The mechanism proposed earlier for CdS sensitized Ag-ZnO catalyst [34] may successfully explain the results observed here. Equations (1)–(4) explain the catalytic activity of the anthocyanin sensitized ZnO particles described in Scheme 1.



where, Anth refers to anthocyanin; e^-_{CB} refers to electron injected in the ZnO conduction band; and Contam refers to organic compounds resulting from bacterial death.

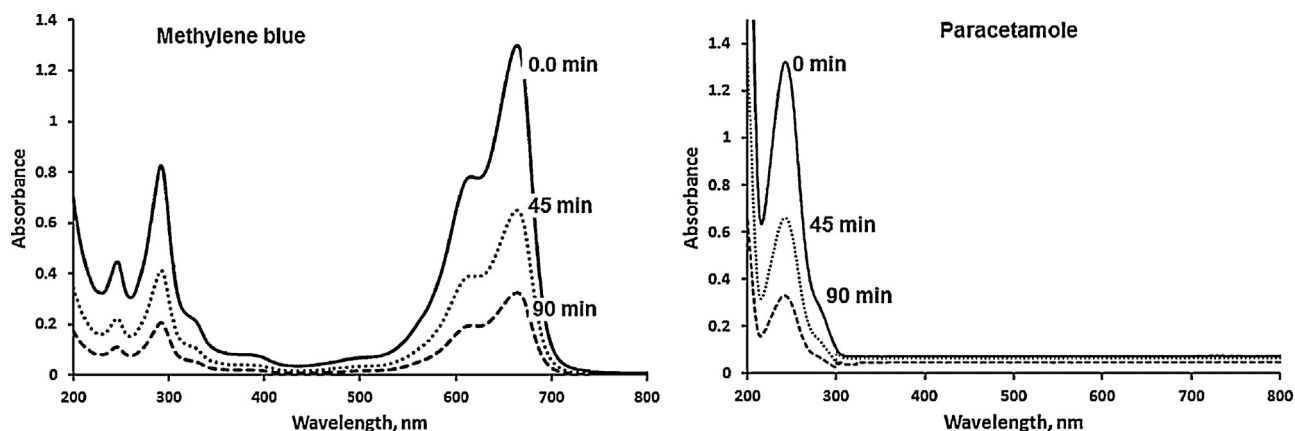


Fig. 13. Spectral analysis of reaction mixtures showing absorbance vs. time throughout photo-degradation of methylene blue and paracetamol. Both reactions were performed using the sensitized ZnO (0.1 g) mixed with aqueous solution (50 mL) of contaminant (10 ppm) at room temperature. Solar simulated light was used with intensity (0.0102 W/cm²).

Scheme 1 and equations (1)–(4) are justified by the energetics of the ZnO and the anthocyanin molecules. As discussed above, the band gap for anthocyanin is ~ 2.1 eV, which is equivalent to 590 nm (visible). This means that the anthocyanin molecules can be readily excited by the visible light. The LUMO level for anthocyanin is reported to have values in the range -0.5 V (in HCl medium) to -0.4 V (with no HCl) with respect to NHE reference [54]. Both values are higher (more negative) than the lower edge of CB for ZnO (~ -0.30 V). Therefore, the injection of the excited electron from the anthocyanin LUMO level to the ZnO CB is possible.

On the other hand, the HOMO level for anthocyanin molecule is $\sim +1.3$ V (in HCl medium) and up to $\sim +2.5$ V (with no HCl) with respect to NHE electrode [54]. Both values for anthocyanin HOMO level are positive enough to cause oxidation for organic contaminants in water which is a thermodynamically feasible process as discussed above. The reported HOMO level ($+2.5$ V) is positive enough to oxidize H₂O molecules into the highly active OH[•] radicals, which (together with oxygen) oxidize different organic molecules in water. The oxygen molecules can also be reduced to yield the active superoxide O₂⁻ ions (equation (3) above) that also react with H⁺ ions to yield the highly active OH[•] radicals [16,52,53]. This mechanism is different from that discussed above, as it involves oxidation of water. Based on reported HOMO levels it cannot be ruled out.

Work is underway to support the sensitized ZnO particles on different surfaces to make their recovery and reuse for multiple times possible. Mineralizing different types of bacteria, especially the stable gram+ positive ones should also be examined.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.jphotochem.2016.05.020>.

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