

Article

Disinfection of Water Borne Pathogens *Escherichia coli* and *Staphylococcus aureus* by Solar Photocatalysis Using Sonochemically Synthesized Reusable Ag@ZnO Core-Shell Nanoparticles

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Abstract: Water borne pathogens present a threat to human health and their disinfection from water poses a challenge, prompting search for newer methods and newer materials. Disinfection of Gram-negative bacterium *Escherichia coli* and Gram-positive coccal bacterium *Staphylococcus aureus* in aqueous matrix was achieved within 60 and 90 minutes respectively at 35°C using solar-photocatalysis mediated by sonochemically synthesized Ag@ZnO core-shell nanoparticles. The efficiency of the process increased with increase in temperature and at 55°C the disinfection could be achieved in 45 and 60 min respectively for the two bacteria. A new ultrasound assisted chemical precipitation technique was used for the synthesis of Ag@ZnO core-shell nanoparticles. The characteristics of the synthesized material were established using physical techniques. The material remained stable even at 400°C. Disinfection efficiency of the Ag@ZnO core-shell nanoparticles was confirmed in case of real world water samples from pond, river, municipal tap and was found to be better than that of pure ZnO and TiO₂ (Degussa P25). When the nanoparticle based catalyst was recycled and reused for subsequent disinfection experiments, its efficiency did not change remarkably even after three cycles. The sonochemically synthesized Ag@ZnO core-shell nanoparticles have a good potential for application in solar photocatalytic disinfection of water borne pathogens.

Keywords: core-shell; disinfection; *Escherichia coli*; nanoparticles; pathogens; silver; solar-photocatalysis; *Staphylococcus aureus*; water; zinc oxide

1. Introduction

A large population of the developing countries is vulnerable to water born diseases caused by pathogenic microbes present in the aquatic environment. Amongst various enteric pathogens, *Escherichia coli* and *Staphylococcus aureus*, are causal agents of various types of infections [1] and may lead to deterioration in the quality of drinking water in rural habitation. The major cause behind these shall be specified to unawareness about personal hygiene practices and poor sanitation facility. For disinfection of these, composite nanoparticles assisted photocatalysis can have a good potential for field application [2]. Currently, the simplicity and cost-effectiveness of sun-light assisted photocatalysis by using metal/metal oxide nanoparticles is gaining much attention for water treatment applications [3]. However, although the nanosized catalysts were successfully developed, certain issues such as short-shelf life of the nanoparticles system due to catalyst poisoning, decrease

in the active surface area of nanoparticles system by surface doping, and possibility of leaching of reactive metal ions to resultant water have restricted their commercial exploitation. To deal with these limitations, core-shell structure nanoparticles were proposed. It is expected that, nanoparticles with core-shell morphology will not only protect the metal catalysts but also show promising results with regards to increased photocatalytic disinfection efficiency and extended shelf life of the material [4]. Metal@ZnO core-shell structure nanoparticles have been used for photocatalytic degradation of organic dyes Rhodamine B and methyl orange in an aqueous solution [5-6]. *E. coli* has been extensively used as a good model micro-organism for studying photocatalytic disinfection but studies with *S. aureus* are mostly done with TiO₂ and its doped variants [7-8]. As far our knowledge, no such disinfection with core-shell nanoparticles has been carried out with the later micro-organism. Ag/TiO₂ nanocomposites were previously explored for successful photocatalytic disinfection of *E. coli* [9]. Most of these reports have followed precipitation technique for coating metal oxide shell on Nobel metal (e.g. gold) nanoparticles. Although these materials have shown interesting photocatalytic property, the high cost of gold is expected to hinder their practical application. Hence, Aguirre et. al tried to replace gold core by cheaper alternative i.e. silver and used this material for degradation of dyes [10]. Das and co-workers for the first time applied Ag@ZnO nanoparticles synthesized by a chemical precipitation technique for sun-light assisted photocatalytic disinfection of a pathogenic bacterium *Vibrio cholerae* in synthetic as well as real water systems [11]. This could be a potential alternative to conventional disinfection techniques such as chlorination which are known to generate toxic byproducts [12]. However the conventional precipitation technique employed for the synthesis of metal@ZnO core-shell nanoparticles could not provide well dispersed and porous materials required for catalytic applications [13]. Thus, it was necessary to investigate alternative synthetic protocols to obtain the monodispersed metal@ZnO core-shell nanoparticles and to check the potential of such materials for photocatalytic applications. Recently, sonochemical techniques have been used extensively to obtain well dispersed and highly crystalline nanomaterials [4,14]. However, to the best of our knowledge, such techniques have never been exploited for the synthesis of metal@ZnO core-shell nanoparticles and examining their potential to disinfect bacterial pathogens. In the present paper, we report sun-light assisted photocatalytic disinfection of two water borne pathogenic bacteria *Escherichia coli* and *Staphylococcus aureus* in saline solution (0.9%) and real water systems using Ag@ZnO core-shell nanoparticles synthesized using an ultrasound assisted method.

2. Materials and methods

2.1. Materials

All the reagents and chemicals used in the synthesis of photocatalyst as well as in the disinfection reaction were of research grade (99.99% pure) and were procured from MERCK, India. DI water was used during all synthesis processes.

2.2. Synthesis and Characterization of Ag@ZnO Core-Shell Nanoparticles

Ag nanoparticles were synthesized by reduction of silver perchlorate monohydrate by NaBH₄ and trisodium citrate dihydrate [11]. A transparent bright yellow color was observed immediately due to the formation of the Ag nanoparticles. This colloid after aging for 12 h at room temperature were coated with zinc oxide via an ultrasound assisted precipitation technique until faded brown slurry is obtained [Appendix A]. The synthesized material was washed thrice and oven dried followed by sintering at 200°C and 400°C for 1h. The materials were sent for characterization. [Details mentioned in Appendix A].

2.3. Preparation of Bacterial Cultures

Bacterial strains of the gram negative bacteria *E. coli* DH5-alpha and gram-positive bacteria *S. aureus* were used as the target microorganisms in this study. The strains were purchased from microbial type culture collection and gene bank (MTCC), India. The strains were grown aerobically

in a Nutrient broth (HiMedia, India) at 37°C in a shaking incubator (Labtech, India) at 200 revolutions per minute (rpm). At optical density (OD₆₀₀) 0.6 for *E. coli* and 0.8 for *S. aureus*, corresponding to 10⁸ CFU/ml (CFU = colony forming unit), the bacteria were harvested by centrifugation at 5000 rpm for 10 min. They were thereafter washed with 0.9 % normal saline solution (NSS) to provide appropriate osmotic conditions [11]. All the glassware and plastic-ware used for media preparation, experimental purposes and analysis were sterilized by autoclaving at 121°C, for 20 min before being used [15].

2.4. Photocatalytic Disinfection Experiments

Bacterial cells with a final cell concentration of 5 × 10⁶ CFU/mL were put in 1 L of normal saline solution and multiple reactions were performed with varying concentrations of Ag@ZnO ranging from 1 to 5 mg/L. Photocatalytic disinfection reactions were carried out in 2 L reactor vessels under continuous and controlled agitation (500 rpm). The set up was kept in dark condition for 30 min to attain equilibrium. After the dark phase, the system was exposed to sun-light for 120 min and samples were collected at 15 min interval. To monitor and analyze the inactivation of microbes, 100 µL of collected samples were further diluted in 900 µL of sterile 0.9% NSS and a volume of 100 µL from the final diluted sample was spread on nutrient agar plates. The plates were left for overnight incubation at 37°C. Following this, viable cell count was performed to obtain the results for the rate of disinfection [11,16]. The above steps were repeated using two commonly used catalysts ZnO and TiO₂ (Degussa P25) for comparative studies and with the optimum catalyst concentration for proper disinfection as obtained by Ag@ZnO. Additionally two experimental controls were performed. 1) In light control, under only photolytic condition the microbial population was exposed to sun-light in absence of Ag@ZnO. 2) In dark control, microbial population was reacted with Ag@ZnO in absence any light. To evaluate whether the sun-light/Ag@ZnO assisted photocatalytic disinfection system is applicable to natural water systems, samples of tap (municipal supply), river, and pond water were collected. Results were compared with de-ionized water. All water samples were collected and transported in clean and autoclaved sample bottles (Tarson) at 4°C and immediately were filtered by using Whatman filter paper and centrifuged at 5000 rpm, 15min to remove insoluble materials followed by autoclaving to eliminate any microbial contamination. To demonstrate the efficiency of the synthesized catalyst, a calculated amount (as mentioned earlier) of targeted pathogens were spiked in the sterilized natural water samples and subjected to photocatalysis in presence of three different photocatalysts (Ag@ZnO, ZnO and TiO₂). The concentration of catalyst used was the one which was obtained as the optimum for the respective bacteria from experiments conducted in saline solution.

2.5. Determination of Lipid Peroxidation

Malondialdehyde (MDA) is an end product of lipid peroxidation. Therefore estimation of MDA through its reaction with thiobarbituric acid (TBA), forming a pink colored MDA-TBA complex, predicts the disintegration rather damage of microbial cell membrane leading to death [16-18]. To establish this analysis was performed by obtaining 1mL samples from the reactor at time intervals (5 min) and the samples were mixed with 2mL of 10% (wt/vol) trichloroacetic acid. The mixture was subjected to centrifugation at 11000 g for initial 35 min and then again for an additional 20 min to ensure the removal of precipitated proteins, catalyst, cells and other possible solid components from the system [16]. 3 mL of freshly prepared 0.67 % (wt/vol) TBA (Sigma Aldrich) was added to the supernatant. The samples were boiled in a water bath for 10 min and then the absorbance was measured at 532 nm using UV-visible spectrophotometer (Shimadzu UV-1800). The concentration of MDA in the system was calculated in nanomoles of MDA released per mg dry weight of bacteria [18].

2.6. Potassium Ion (K⁺) Leakage Studies

To study the K⁺ leakage from photocatalytically inactivated bacteria, 2 mL sample was collected at regular time intervals (2 min) from the reaction system and was subjected to centrifugation as per

the details given in previous reports [18-19]. The supernatant was collected and analyzed using microwave plasma atomic emission spectrometer (4200 MP-AES, Agilent Technologies).

2.7. Stability and Reusability of the Photocatalyst

The stability of the catalyst in post reaction condition was investigated using XRD. Additionally for further confirmation the post reaction water sample was analyzed using MP-AES to detect the leaching of Ag^+ and Zn^{2+} ions during the photocatalytic disinfection experiment [11]. Catalyst was recovered by centrifugation at 10000 g for 30 min and dried at 80°C and reused for the photocatalytic disinfection application.

Unless otherwise mentioned all the experiments were conducted in triplicate.

3. Results and Discussion

3.1. Photocatalytic Disinfection of Target Pathogens

Figure1 (a and b) shows the photocatalytic disinfection achieved against the target pathogens at different catalyst concentrations. In Figure1 (c and d) bacterial disinfection is represented in its corresponding log reduction profile and the disinfection pattern is validated through comparison of the obtained profile with the standardized Chick-Watson model [11,20-21]. Figure1 (a) and (b) suggests that amongst the concentration tested, 2 mg/L and 3mg/L resulted in complete disinfection (6 log reductions) of *E. coli* and *S. aureus* respectively in 60 min and 90 min respectively. It is observed that, only sun-light is not effective in complete disinfection of the targeted pathogens as only 3 and 2.5 log reductions could be observed for *E. coli* and *S. aureus* respectively at 120 min. Experiments conducted in the dark condition did not show remarkable change in the microbial colony count as less than 0.5-log reduction for both the microorganisms was achieved in 120 min [Figure1 (c) and (d)]. Using the optimum concentration of Ag@ZnO nanoparticles for each of the bacteria for photocatalytic disinfection, comparative sun-light assisted photocatalytic disinfection activity was evaluated with pure-ZnO and commercial TiO_2 (Degussa P25) and the results are shown in Figure 2 (a and b). Figure 2 (c and d) shows the Chick-Watson disinfection kinetics of *E. coli* and *S. aureus* using different photocatalysts. These results suggest the superior disinfection efficiency of Ag@ZnO nanophotocatalyst than conventional metal oxide system. An increase in inactivation for both targeted bacteria was observed with increase in catalyst concentration from 1 to 2mg/L in *E. coli* and 1 to 3 mg/L in *S. aureus*. With further increase in the catalyst concentration from the mentioned range, deterioration in disinfection rate was obtained for both the targeted microorganisms. With lower concentration of catalyst the amount of reactive oxygen species (ROS) generated is comparatively less. Thus complete disinfection required longer irradiation time [22]. It is expected that as the rate of ROS production is slow at lower concentration of catalyst and hence at initial conditions the microorganisms may activate their molecular resistance mechanism. Therefore an extended disinfection time period is required for sufficient ROS generation and thus under the constant attack of ROS, bacteria may lose their capability of reactivation. With an increase in catalyst concentration the ROS generation rate increases, which is expected to improve the disinfection rate. Similarly, at the optimal condition the rate of ROS generation is, maximum and therefore it may be expected that, the interaction of the same with bacterial cells is more frequent. This may lead to an enhanced disinfection rate. It is further noticed that with increase in the catalyst concentration, disinfection gets delayed. This is mainly because with increase in catalyst concentration the turbidity of the system increases, thereby blocking the irradiation from sun-light to uniformly reach the catalyst particles and cells hence resulting in slower inactivation [23]. The current study involves *E. coli* and *S. aureus* bacteria. The photocatalytic performance of a photocatalyst depends both on its concentration and the irradiation time. *E. coli* was found more sensitive to sun-light assisted photocatalytic disinfection process than *S. aureus*. As it requires comparatively less catalyst concentration and shorter time of sun-light irradiation in comparison to *S. aureus* as evidenced from Figure 1 (a and b). The difference in susceptibility of both bacterial species to Ag@ZnO Nanoparticles can be ascribed to the variation

in cell membrane/wall structures, chemical components, biological shape, differences in robustness of Gram-positive and Gram-negative bacteria [24].

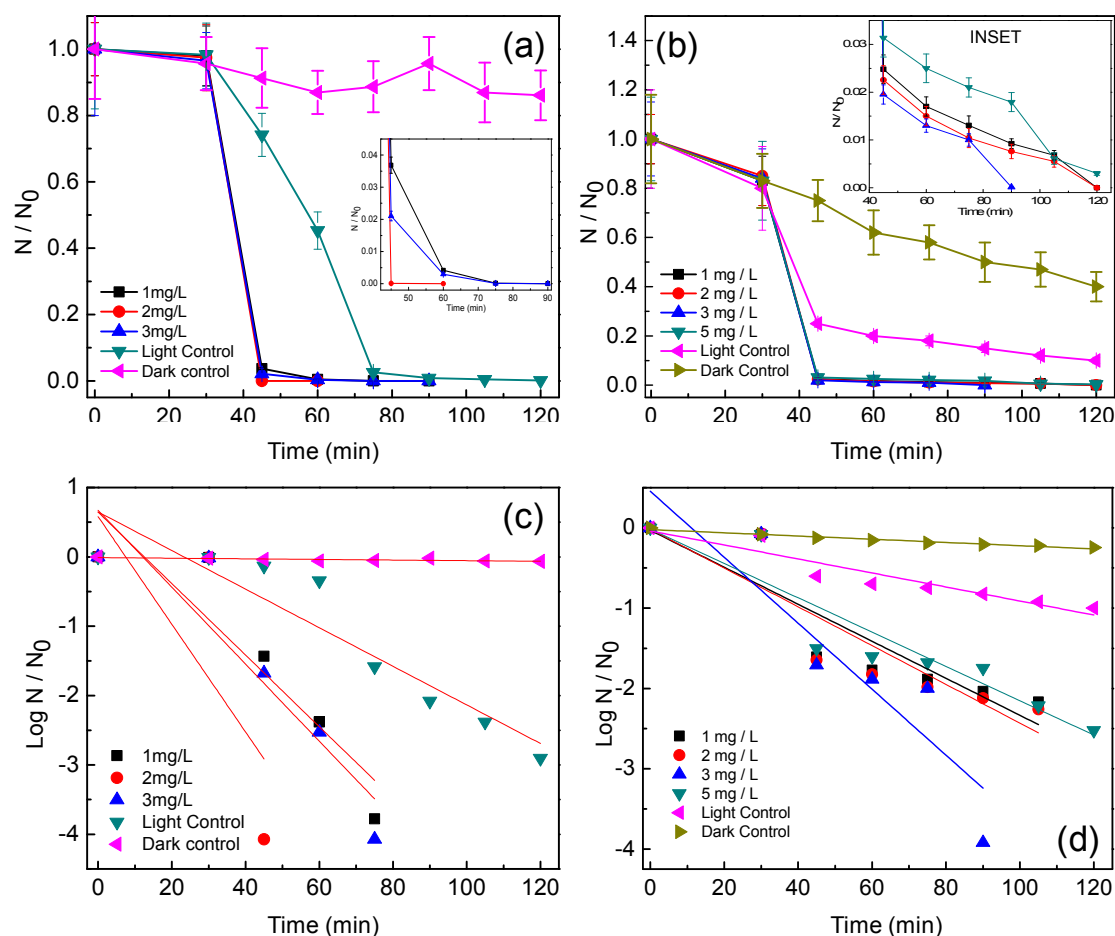


Figure 1. Effect of Ag@ZnO core-shell NPs loading on the solar-PCD kinetics of (a) *E. coli* and (b) *S. aureus*. Linear fitting plots of PCD kinetics of (c) *E. coli* and (d) *S. aureus* according to Chick-Watson model. Initial bacteria concentration = 5×10^6 CFU/mL, Temperature = $35 \pm 2^\circ\text{C}$. Error bars indicate the standard deviation of replicates ($n=3$).

From Figure 2, it is observed that Ag@ZnO Nanoparticle shows enhanced disinfection efficiency for both targeted pathogens in comparison to the classical metal oxide systems (ZnO and TiO₂). The expected reason behind the enhanced efficiency may be the positioning of the Noble metal in the core and encapsulating it with ZnO shell. Photocatalytic disinfection involves the excitation of photocatalyst with light energy greater than or equal to that of the band gap [25]. On excitation the electrons from the valence band of the metal oxide shuttles to the conduction band, where it is usually accepted by electron acceptors present in the reaction environment. This reduction pathway leads to the formation of ROS which results in killing of microbial cells by damaging the membrane integrity [24,26]. Therefore it leads to subsequent release of the intra-cellular components which become vulnerable to the ROS attack [26,27]. Fig 3 (a and c) shows the effect of temperature on photocatalytic disinfection of targeted pathogens. These results show that as the temperature increased maximum process efficiency was observed at reaction temperature of 55°C . It is observed that with increase in the temperature of the reaction system, the rate of disinfection improved. At 55°C disinfection is achieved within 45 min and 60 min for *E. coli* and *S. aureus* respectively. The post disinfection reactivation of the target microbes were assessed for 24h. None of the microbes showed reactivation thus suggesting the cell death by damage caused by the ROS to both the target pathogens.

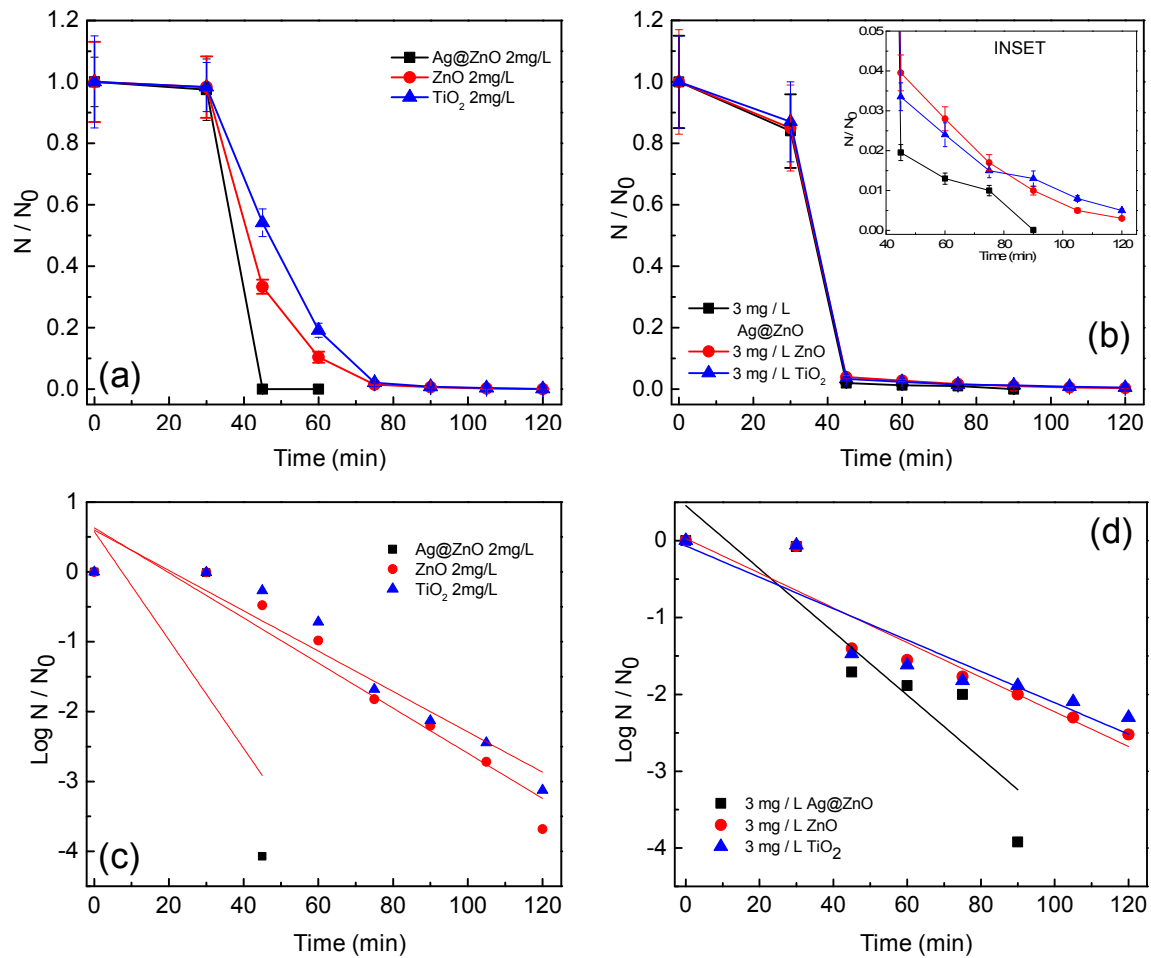


Figure 2. Effect of different catalysts on the solar-PCD kinetics of (a) *E. coli* and (b) *S. aureus* at a catalyst loading of 2 mg/L and 3 mg/L respectively. Linear fitting plots of PCD kinetics of different catalysts against (c) *E. coli* and (d) *S. aureus* according to Chick-Watson model at a catalyst loading. Initial bacteria concentration for each experiments = 5×10^6 CFU/mL, Temperature = $35 \pm 2^\circ\text{C}$. Error bars indicate the standard deviation of replicates ($n=3$).

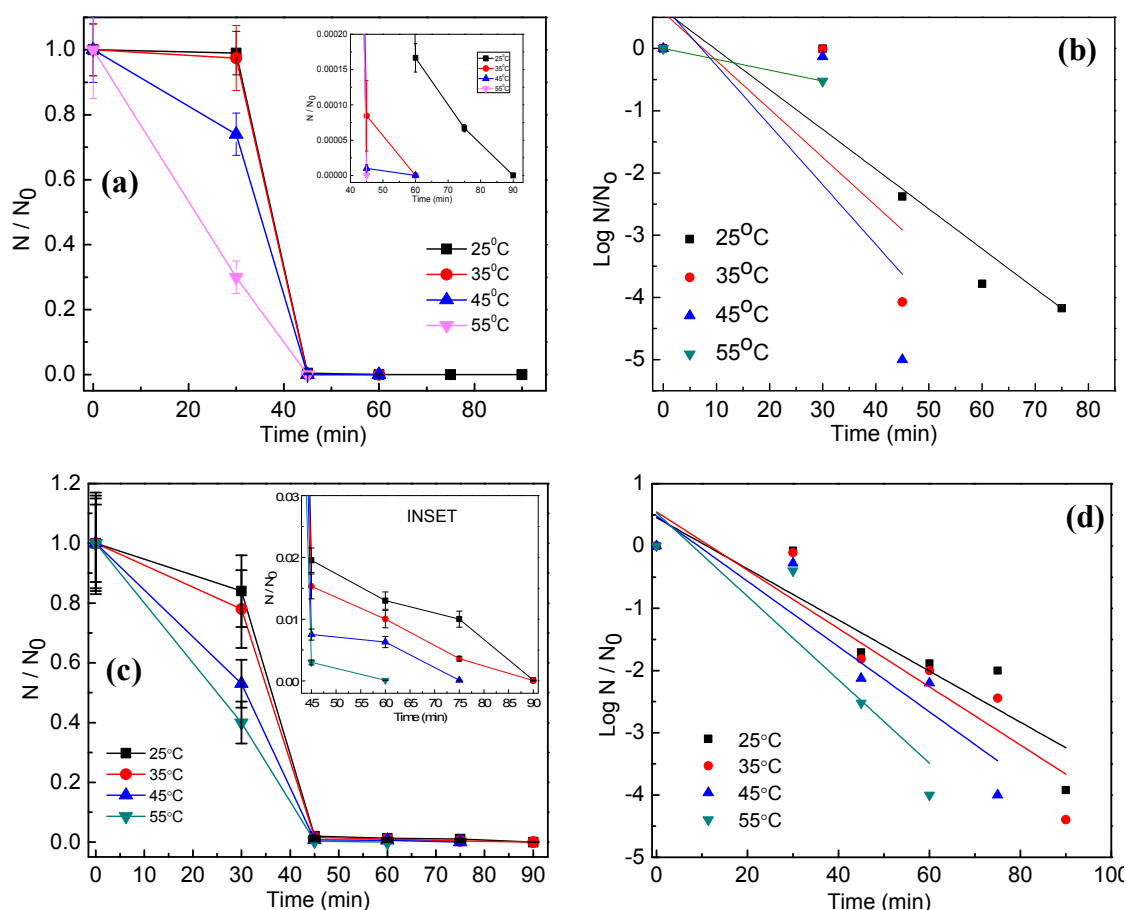


Figure 3. Effect of different reaction temperature on the solar-PCD kinetics of (a) *E. coli* and (c) *S. aureus* at a catalyst loading of 2 mg/L and 3 mg/L respectively. Linear fitting plots of PCD kinetics of different reaction temperature against (b) *E. coli* and (d) *S. aureus* according to Chick-Watson model. Initial bacteria concentration for each experiments = 5×10^6 CFU/mL, Error bars indicate the standard deviation of replicates (n=3)

3.2. Determination of MDA to Study the Membrane Lipid Peroxidation

Time dependent generation of MDA (key biomarker of membrane lipid peroxidation) was determined for *E. coli* and *S. aureus* subjected to photocatalytic disinfection under their respective optimum catalyst concentration and Temperature 35°C is shown in Figure 4 (a and b). Earlier experiments had shown complete disinfection at 60 and 90 min respectively for *E. coli* and *S. aureus* (Figure 1 a and b). Hence, a similar correlative result can be inferred from the above mentioned figure. It is quite evident that maximum generation of MDA is observed after 75 min i.e. 0.03 nmol/mg cell dry weight and 90 min i.e. 0.0375 nmol/mg cell dry weight for *E. coli* and *S. aureus* respectively, which indicates cell membrane disintegration resulting in disinfection. Slight elevation in MDA production is seen within the first 30 min which may be attributed to the loss of membrane integrity due to action of shear stress produced on the microbial cells due to the continuous stirring condition [28]. Additionally, the misbalance of ionic potential may also play a role in loss of membrane integrity leading to peroxidation of lipid. It may also be noticed that after the reported disinfection time, a decline in MDA concentration has been initiated. After a threshold level of MDA is generated in the photocatalytic system, it is expected to be mineralized being an organic compound itself [18,29]. When the microbial cells were exposed to sun-light without the presence of photocatalysts, lesser than even 0.01 nmol/mg cell dry weight generation was observed in both the microbes as shown in Figure 4 (a and b). However, the effect of Ag@ZnO on microbial cells without the presence of light is also found to be non-substantial where concentration was less as 0.005 nmol/mg cell dry weight were quantified for both the test microbes. It is proposed that generation of ROS (such as OH^\bullet radical)

in the photocatalytic process may lead to peroxidation of the cell membrane peptidoglycan layer and membrane proteins followed by decomposition of cellular components and cellular disintegration [16-19,29], as ROS mainly ($\bullet\text{OH}$) hit unsaturated membrane lipids to make lipid radical. This, in presence of oxygen is expected to give a lipid peroxy radical capable of abstracting hydrogen from the adjacent unsaturated lipid and produce lipid hydroperoxide and a lipid radical. This reaction series continues until all the membrane unsaturated lipids are destroyed and malondialdehyde (a stable by-product of membrane lipid peroxidation) is subsequently produced. MDA generation pattern suggests that lipid peroxidation in *E. coli* maintains a uniform rate while a sporadic rate is obtained for *S. aureus*, thus suggesting the robustness of the later microbe in comparison to the former [24].

3.3. Analysis of Potassium Ion (K^+) to Study the Cell Membrane Damage

Leakage of K^+ ion is generally considered as a dominating evidence of cellular compromise. The results obtained through K^+ leakage analysis are in agreement with many previous studies which mention about the dysfunction of potassium channels of microorganism on photocatalytic treatment [18, 30-32]. It can be observed that the concentration of K^+ (in ppm) increases with increase of the reaction time up till a particular time period beyond which the concentration becomes constant in the reaction environment. As shown in Figure 5 (a and b) the maximum K^+ estimated after 120 min for *E. coli* and *S. aureus* after photocatalytic disinfection was found to be 575ppb and 440 ppb respectively. An interesting observation was made that the time required for complete disinfection for each of the target bacteria, as evaluated from the decreasing CFU count does not correspond well with the pattern of K^+ leakage. This must be because, the primary target of photocatalytic produced ROS is membrane lipids. Once the entire membrane of bacteria is compromised, an increase in K^+ ion is expected. This pattern of K^+ release suggests that increase in the concentration of potassium ion in the reaction environment indicates a steady progress in the photocatalytic disinfection process. Once the entire bacterial death is achieved, it is expected that the total amount of K^+ will be maintained for the remaining reaction phase [33].

3.4. Stability and Reusability of the Catalyst Post Disinfection

When the stability of the catalyst in post reaction condition was investigated using XRD, no alteration in crystal structure of Ag@ZnO was observed, suggesting its structural stability throughout the process [11, 34]. It is known that leaching of material could re-toxify the system and it could also be argued that Ag^+ and Zn^{2+} ions which are reported to show antimicrobial property may leach out of the system and hence, may be the actual cause of disinfection. However, the answer to this possibility is already communicated in our previous paper [11], there being no detectable amount of Ag^+ and Zn^{2+} ions in the system post disinfection. If the catalyst could be recycled after photocatalytic disinfection then it may be suitable for commercial exploitation of the process. Ag@ZnO core-shell Nanoparticles were recycled after photocatalytic disinfection experiment and used for next batch of bacterial disinfection experiment (after heating at 80°C). As shown in Figure 7, core-shell nanophotocatalyst exhibited insignificant reduction in *E. coli* and *S. aureus* disinfection efficiency even after three consecutive cycles.

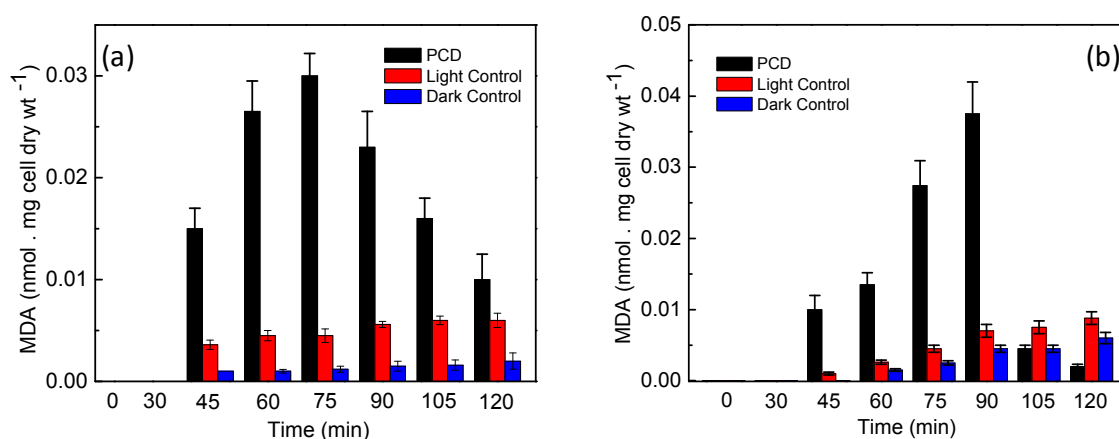


Figure 4. Lipid peroxidation kinetics of (a) *E. coli* and (b) *S. aureus* cells subjected to solar-photocatalysis in presence of 2mg/L and 3mg/L Ag@ZnO NPs respectively. Initial bacteria concentration = 5×10^6 CFU/mL, Temperature = $35 \pm 2^\circ\text{C}$. Error bars indicate the standard deviation of replicates (n=3).

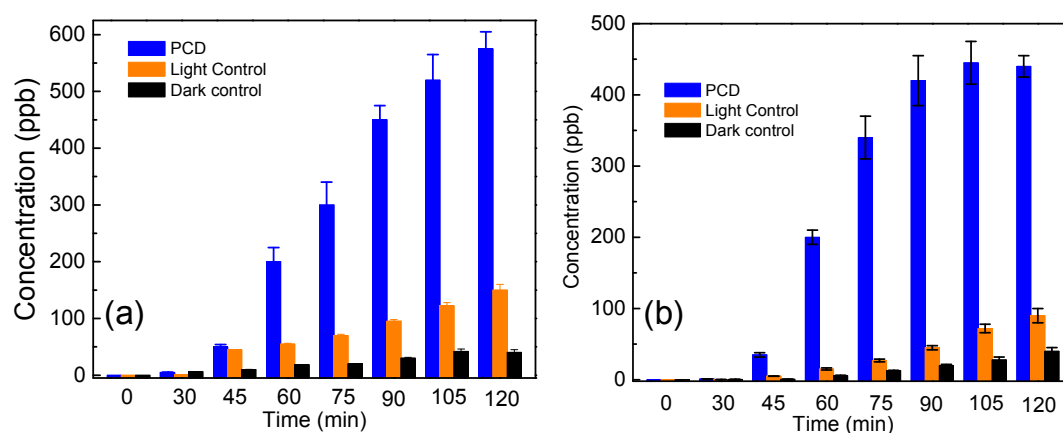


Figure 5. Leakage of K⁺ ion from (a) *E. coli* and (b) *S. aureus* cells subjected to solar-photocatalysis in presence of 2 mg/L and 3mg/L Ag@ZnO core-shell NPs respectively. Initial bacteria concentration = 5×10^6 CFU/mL, Temperature = $35 \pm 2^\circ\text{C}$. Error bars indicate the standard deviation of replicates (n=3).

3.5. Photocatalytic Disinfection Efficiency in Real Water Systems

As the results show (Figure 7), Ag@ZnO exhibits a better disinfection profile as compared to pure semiconductors in case of all the real water samples. The results correspond well with our previous results [11]. Many causes can be attributed to the superiority of the Ag@ZnO as compared to the traditional photocatalysts. It is a well-known and established fact that the photocatalyst that is being used here has a Core Shell Nanocomposite structure. The structure itself has many advantages over its traditional counterparts. It is a matter of general observation that the metal ions in the composite structure are protected in the form of a shell in the composite structure. This has many advantages; firstly it solves the problem of leaching out of the Silver metal ion. Silver is itself a very poisonous metal ion and detrimental and harmful to various organisms [4-5,11]. At the same time, the target pathogens of *E.coli* and *S.aureus* are unable to survive and escape its wrath. The core shell morphology also increases the surface area of the photocatalyst. As the surface area increases, so does the effectivity of a certain photocatalyst increases. Both the traditional photocatalyst that are used here, namely TiO₂ and ZnO lack in this aspect. The lack of a proper Nano-Composite structure in the cases of TiO₂ and ZnO can also be attributed to the less efficiency that these photocatalysts show in the aspects of both Photocatalytic degradation in case of real water samples.

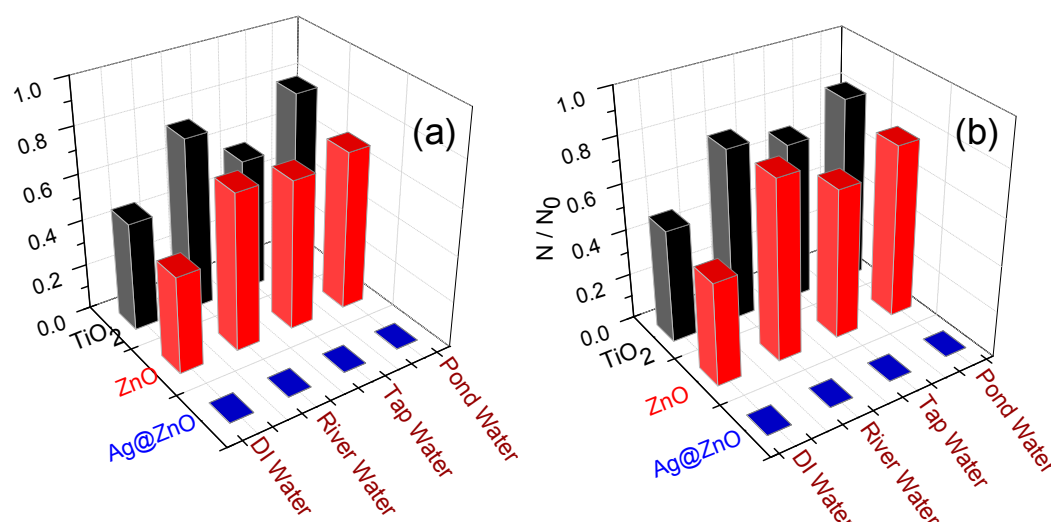


Figure 6. Effect of different photocatalysts on the relative reduction in the (a) *E. coli* and (b) *S. aureus* cell count (N/N_0) in real water samples after 120 min of solar irradiation at a catalyst loading of 2mg/L and 3mg/L catalyst concentration respectively. In each case the initial bacteria concentration = 5×10^6 CFU/mL, Temperature = $35 \pm 2^\circ\text{C}$.

Various studies have already shown that both Zinc and Silver are detrimental to the growth of microorganisms, in various concentrations [35]. The photocatalyst that we have used, contains both the elements, so as a result, a better result can always be expected than that from the traditional ones, namely the likes of ZnO and TiO_2 . The combined effect of toxicity of these two potent antimicrobial agents, combined with the lesser amount of leaching due to the unique structure is indeed a deciding factor in increasing the efficiency of the photocatalyst against the traditional players [11].

However, the issue of safety can be raised, regarding the compatibility of Silver and Zinc in various water streams and water bodies, as both of these metals are known to be toxic to organisms [36–37]. To attend these sensitive issues, we did an MP-AES assay, and it was observed that the concentration of Zinc and Silver was below the detectable levels. This it solves most of the toxicity related issues.

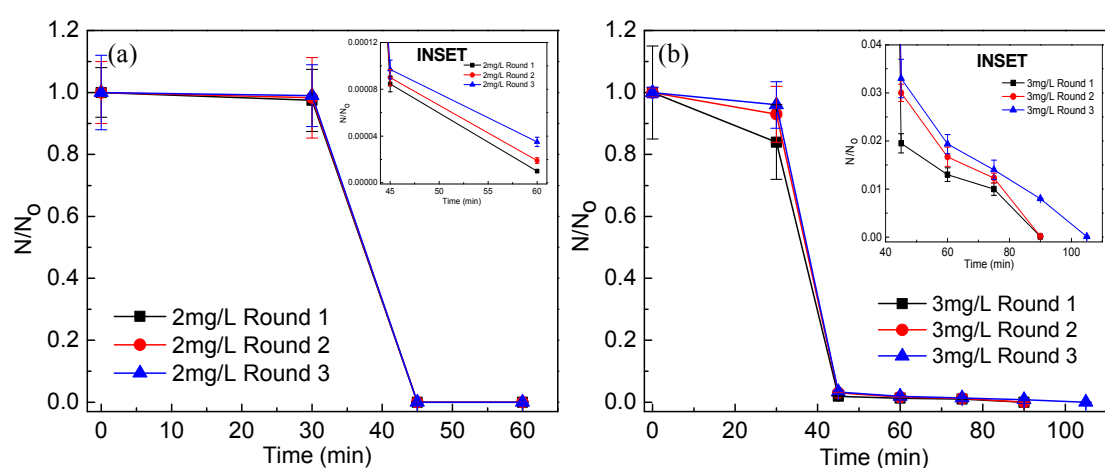


Figure 7. Effect of Ag@ZnO core-shell NPs reusability till 3 rounds of solar-PCD kinetics of (a) *E. coli* and (b) *S. aureus*. Initial bacteria concentration = 5×10^6 CFU/mL, Temperature = $35 \pm 2^\circ\text{C}$. Error bars indicate the standard deviation of replicates ($n=3$).

There is an increasing demand regarding the issue of providing safe and potable drinking water to a large chunk of the underdeveloped persons that reside in the third world countries. There is an urgent need to develop strategies that follow an alternate route to address this concern [18]. Based on the above statement, it will be conspicuous to propose the above mentioned concept of “Advanced Oxidation Process” and the catalyst that we have proposed, based on the proven effectivity and superiority as compared to that of other traditional catalysts that have been discussed here.

The catalyst that we have proposed, is generally working well towards the basic ranges of pH. All the real water samples, especially the likes of tap-water, river water have basic pH. This can also be attributed towards the better effectivity and working efficiency of the proposed catalyst, although better elucidation is required.

It can also be concluded from the MP-AES analysis that the proposed catalyst is completely non-toxic in nature and can be applied for a wide range of applications. It can also be concluded that, since there is no evidence for the proposed catalyst's toxicity to organisms, it can surely be used as a better, safer option than the traditional ones.

4. Conclusion

When DI water contaminated with *E. coli* and *S. aureus* was subjected to Ag@ZnO core-shell nanoparticles mediated photocatalytic disinfection under sun-light radiation, complete disinfection of *E. coli* and *S. aureus* was achieved within 60 and 90 minutes respectively at 35°C and in 45 and 60 min at 55°C. Quantitative analyses of K⁺ ion release and MDA assay proposed the damage of bacterial cell wall by ROS generated during solar-photocatalysis. The disinfection profile for both the bacteria was validated using Chick-Watson disinfection model. Disinfection achieved using the Ag@ZnO system was also validated for real world water samples from municipal tap, pond and river. When the nanocatalyst was recycled and reused for subsequent photocatalytic disinfection experiments, its efficiency did not change remarkably even after three cycles. The communicated photocatalytic system may find its application in designing a portable water decontamination system at pathogen infested geographical locations.

Supplementary Materials: The following are available online at www.mdpi.com/link, Figure S1: UV-Visible spectra of aqueous dispersion of Ag and Ag@ZnO nanoparticles synthesized by chemical reduction and sonochemical technique respectively, Figure S2: XRD patterns of the Ag@ZnO core-shell nanoparticles synthesized by the sonochemical technique and heat treated for 2h at different temperatures, Figure S3: FTIR spectrum of Ag@ZnO core-shell nanoparticles synthesized by the sonochemical technique and dried at 80°C for 2 h, Figure S4: Room-temperature photoluminescence spectrum of Ag@ZnO core-shell nanoparticles synthesized by the sonochemical technique and dried at 80°C for 2 h, Figure S5: TEM (a) and HRTEM (b) images of Ag@ZnO nanoparticles synthesized by the sonochemical technique and dried at 80°C for 2 h, Figure S6: Nitrogen adsorption/desorption isotherms obtained at 77 K and inset shows the pore size distribution of the as-synthesized Ag@ZnO nanoparticles synthesized by the sonochemical technique and dried at 80°C for 2 h.

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Author Contributions: Suraj K. Tripathy created the original study plan. Sourav Das, Neha Ranjana and Ananyo Jyoti Misra designed and executed the experiments under the guidance of Suraj K. Tripathy. Amrita Mishra and Mrutyunjay Suar helped in the molecular biology experiments. Cecilia Stålsby Lundborg and Ashok J. Tamhankar reviewed and edited the manuscript. All authors read and approved the manuscript.

Conflicts of Interest: The authors declare no conflict of interest.

Appendix A

Experimental Details for Synthesis of Ag@ZnO Core-Shell Nanoparticles

Ag nanoparticles were synthesized by reduction of Silver perchlorate monohydrate (Sigma-Aldrich) by NaBH_4 and trisodium citrate dihydrate. Experimental procedures were as follows: 97 mL of distilled water was placed in a 250 mL glass beaker which was placed in an ice bath. 1 mL of silver perchlorate monohydrate (1 mM) followed by 1 mL of 100 mM sodium borohydride and 0.885 mL of 3 mM of trisodium citrate were added to the beaker under vigorous stirring. A transparent bright yellow color was observed immediately due to the formation of the Ag nanoparticles. This colloid was aged for 12 h at room temperature. Zinc oxide nanoparticles were coated on the surface of Ag nanoparticles via an ultrasound assisted precipitation technique. To 50 mL of zinc nitrate hexahydrate aqueous solution of a known concentration, sodium hydroxide solution (1 M) was added to obtain a white precipitate of zinc hydroxide, which was re dissolved by adding excess of sodium hydroxide. 20 mL of this solution was added to 10 mL of aqueous dispersion of Ag nanoparticles and exposed to ultrasound for 30 to 90 min. Then the solution was allowed to cool by a natural process. The composite nanoparticles were then collected by centrifugation (at 12000 rpm) and dried at 80°C for 12 h. Following this, the nanoparticles were sintered at 200°C and 400°C for 1h. During centrifugation nanoparticles were washed with de-ionized water (three times) to remove the water soluble sodium chloride and other impurities.

Materials Characterization

Morphology of the nanoparticles was investigated by TEM (JEOL-JEM-2010). TEM samples were processed by dipping the TEM grid in aqueous dispersion of nanoparticles followed by freeze drying for 12h. The crystal structure of synthesized nanoparticles was studied by using XRD (D/Max 2005, Rigaku). Optical properties were investigated using UV-visible spectroscopy (UV-1800, Shimadzu). Average surface area and porosity was measured by BET (Brunauer–Emmett–Teller) technique. The presence of adsorbed molecules and/or functional groups on surface of Ag@ZnO nanoparticles is analyzed with FTIR spectroscopy at room temperature. Optical property of the Ag@ZnO core-shell structure nanoparticles processed by sonochemical technique is investigated by photoluminescence (PL) spectroscopy with an excitation wavelength of 324 nm.

UV-visible spectra of the aqueous dispersion of Ag nanoparticles, and Ag@ZnO core-shell nanoparticles synthesized by ultrasonic hydrolysis of zinc nitrate hexahydrate are shown in Supplementary Figure S1. Aqueous dispersion of Ag nanoparticles showed a clear SPR band at 391 nm which showed a distinct red shift of about 22 nm immediately after addition of aqueous sodium zincate sol. This is attributed to an immediate change in the chemical environment around Ag nanoparticles. With increase in the ultrasonic irradiation time SPR band has shown a red shift to 397 nm with development of a shoulder peak at 487 nm. It is expected that during the formation of core-shell nanoparticles, Ag nanoparticles may have aggregated slightly to form large clusters. This may have caused dipole coupling between closely interacting metal nanoparticles. This hypothesis is supported by the electron microscopy images (Supplementary Figure S5)

Result of XRD study is shown in Supplementary Figure S2. For the synthesized nanoparticles, three distinct peaks at $2\theta=38.2$, 44.9 and 64.8 corresponding to (111), (200) and (220) planes of metallic Ag with face-centered cubic structure (JCPDS Card No. 04-0783) is observed. Similarly three major peaks of ZnO at $2\theta=31.99$, 34.63, and 46.51 corresponding to (100), (002), and (102) planes of synthetic ZnO with hexagonal wurtzite structure (JCPDS Card No. 36-1451) are obtained. Any peak corresponding to other Ag/Zn compounds was not obtained. This suggests that no alloy or solid solution is formed. Mean crystallite diameter (MCD) was found to be ≈ 15 and 25 nm for Ag and ZnO nanoparticles respectively. It is also observed that the crystal structure and phase remained unchanged after heat treatment (at 200 and 400°C). However the MCD and crystallinity have increased slightly after heat treatment.

The results of FTIR spectroscopy are shown in Supplementary Figure S3. Optical properties obtained by PL spectroscopy are shown in Supplementary Figure S4. Morphology of the Ag@ZnO nanoparticles synthesized by sonochemical technique was investigated by TEM. Supplementary Figure S5 shows simultaneous TEM and FESEM images of core-shell Ag@ZnO nanoparticles. Core-shell structure is observed for the nanoparticles. However, multiple silver nanoparticles were encapsulated within a single zinc oxide shell. A broad size distribution is observed for as synthesized Ag nanoparticles. Size of the Ag nanoparticles is found to be in the range of 10–30 nm and that of ZnO shell is about 6 to 8 nm.

The adsorption-desorption isotherm plot for the nitrogen sorption (77 K) of the Ag@ZnO nanoparticles sample that was synthesized by sonochemical technique and dried at 80°C for 2 h shows typical “type IV” isotherm in the Brunauer classification. Supplementary Figure S6(a) A typical pore size distribution for the nanoparticles is shown in inset of Supplementary Figure S6(b). The sample exhibited average pore size in the range of 5–20 nm indicating the porous nature of the material. The specific surface area of Ag@ZnO core-shell nanoparticles was evaluated to be 65.5 m²/g based on the BET result. This high surface area and porous nature is expected to be very beneficial for photocatalytic applications.

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