

A comparative study of silver nanoparticles and corona discharge for environmental and antibacterial applications

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ORIGINAL RESEARCH ARTICLE

ABSTRACT

We have presented a comparative study of silver nanoparticles (NPs) and corona discharge for decomposition of Eosin Y (EY) as a model dye and bactericidal activity of a series of gram-positive and gram-negative bacteria as representative microorganisms. Despite the fact that silver NPs demonstrate a great antibacterial activity, corona discharge process showed a significant decrease in survived bacteria in a few minutes. Furthermore, the results revealed that corona discharge at ambient air significantly enhanced degradation rate of model dye compared to silver NPs. Dye removal was about 88% after 10 min in corona discharge process compared to 36% dye removal after 2 h in the presence of silver NPs. It was suggested that corona discharge treatment, which is based on the most efficient energetic species, was more powerful than silver NPs in environmental and antibacterial applications which shown potential to kill different bacteria and degrade EY dye in less than 10 min.

KEYWORDS

antibacterial properties; corona discharge; environmental applications; silver NPs

1. INTRODUCTION

Treatment of bacterial and environmental contaminants is the most demanding topic in scientific community due to the universal concerns in recent years. In this regard, many research studies are currently undergoing to identify more efficient procedures for the treatment of bacterial infections and environmental pollutions (Jie et al., 2009; Zhang et al., 2010). Many physical and chemical approaches have been developed for the treatment of such kind of contaminations such as antibiotics, microbiological or enzymatic decomposition, degradation by catalytic materials, advanced oxidation processes (AOPs), etc. (Tao et al., 2005; Bhatnagar and Sillanpaa, 2010; Ellouze et al., 2010; Wang et al., 2010; Barot and Bagla, 2012; Sharma, 2012; Kapoor et al., 2013). Due to the intrinsic disadvantages of chemical and physical methods for the removal of contaminations, the use of

corona discharge method for the treatment of bacteria and chemical pollutants has received much attention as an viable and efficient substitute (Zuo et al., 2003; Mok et al., 2007; Bujacek et al., 2012). On the other hand nanomaterials such as silver NPs have also exhibited potential applications in biotechnology, chemical sensing, photo catalysis, water and air purification and so on.

Several researchers demonstrated that various biomolecules can be efficiently removed by directly exposing the surface to low pressure discharges. Sari and Fadaee (2010) used a low cost air corona discharge at atmospheric pressure and room temperature for inactivation of *Escherichia coli* (*E. coli*) and *Pseudomonas aeruginosa* bacteria. They have found that the colony survival rate decreases exponentially. The authors concluded that corona discharge is an efficient way for decontamination of microorganisms particularly when it couples with an external magnetic

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field. Similarly, Birmingham and Hammerstrom (2000) demonstrated that an atmospheric pressure non thermal plasma can competently deactivate bacteria in gases, liquids, and on surfaces as well as shown potential to decompose hazardous chemicals.

In this work, we have performed a comparative study of silver NPs and atmospheric corona discharge on antibacterial properties and degradation of model dye. Furthermore, the effect of discharge duration on bactericidal efficacy on a series of gram-positive and gram-negative bacteria and degradation rate of EY were evaluated

2. MATERIALS AND METHODS

2.1. Experimental details

For synthesis of silver NPs, 10 mL of AgNO_3 (1 mM) was injected to 30 mL of NaBH_4 (2 mM) through a syringe pump (Ascor AP 22) at a rate of 100 mL/h under vigorous stirring in an ice bath. The reaction mixture was stirred vigorously on a magnetic stir plate for another 30 min. The yellow color of the final solution clearly indicates the formation of colloidal silver NPs.

Corona discharge was produced by a Tesla coil in which a high voltage supply provided by transformer was used to generate the spark gap. Transformers charge the high voltage capacitors to fire the spark gap. Then energy in the primary coil is transferred into the secondary coil by a magnetic coupler which results in an extremely high voltage at the top of the secondary coil which is the last place for the electricity before it jumps into the air. Figure 1 demonstrates the experimental system used in this work. The corona discharge gun works with AC 220–240 V and 50–60 Hz at room temperature (25 °C). The facility has a sharp electrode at the end for boosting the electrical field. In this situation the electrical field is concentrated around the electrode. Figure 1b and 1c show photographs of a typical corona discharge process on EY dye and *E. coli* bacteria, respectively.

2.2. Material characterization

The size distribution of NPs was measured using a zeta series Malvern instrument at room temperature. Scanning electron microscopy (SEM) analysis was carried out by a SEM instrument (Philips XL30) at 5 to 20 keV accelerating energy. Transmission electron microscopy (TEM) analysis was performed using a TEM, PHILIPS EM208 instrument at 100 to 200 keV

accelerating energy by deposition of silver NPs onto the copper grid at room temperature. Optical absorption spectroscopy of silver NPs and degradation rate of EY was probed using a double beam Optizen POP spectrophotometer (Mecasys Company, Korea) in the range of 200 to 1000 nm wavelengths by measuring the maximum absorption peak of model dye at different discharge duration times.

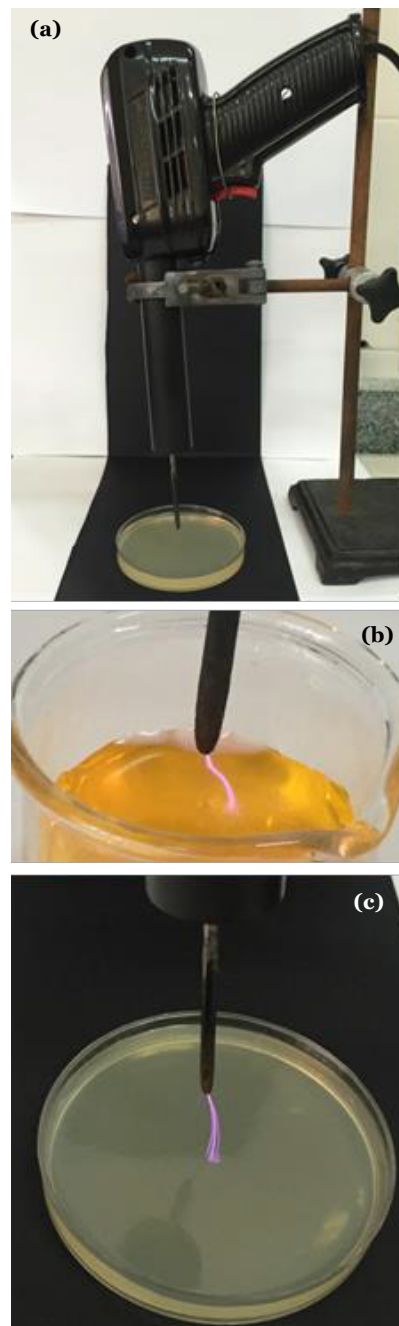


Figure 1. Experimental set up showing the (a) corona discharge facility, (b) corona discharge process for degradation of dye and (c) corona discharge for antibacterial process.

2.3. Bacterial assessments

Bactericidal efficacy of corona discharge method and silver NPs were studied against different gram-positive (*Staphylococcus aureus*, *Micrococcus luteus* and *Bacillus subtilis*) and gram negative (*Escherichia coli*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*) bacteria. Before the microbiological experiments, all glassware and samples were sterilized by autoclaving at 120 °C for 20 min. The microorganisms were cultured on a nutrient agar plate at 37 °C for 24 h. For the antibacterial test, each sample was placed into a sterilized Petri dish. Then 0.1 mL of the diluted saline solution containing a specific type of bacteria was mixed with the prepared sample. After that, the bacteria were washed with 5 mL of phosphate buffer solution in the sterilized Petri dish. Then 1 mL of each bacteria suspension was spread on a nutrient agar plate and incubated at 37 °C for 24 h before counting the surviving bacterial colonies.

3. RESULTS AND DISCUSSION

Physicochemical properties of silver NPs were probed by scanning electron microscopy (SEM), transmission electron microscopy (TEM), dynamic light scattering (DLS) and Ultraviolet-visible (UV-Vis) spectroscopy. Figure 2a shows a typical SEM image of prepared silver NPs. As seen in Figure 2a, nanoscale particles with almost uniform distribution are formed but, the size of the NPs was too small to be well observed by SEM. In this regard, TEM was employed to visualize the shape and size of the obtained NPs (Figure 2b). As it was clear from TEM image, silver NPs with average diameters of about 35 ± 5 nm were formed. To further confirm the size of the silver NPs, DLS analysis was also performed which is depicted in Figure 2c. Size distribution of the particles (repeated 3 times) reveals formation of NPs with average diameter of ~ 40 nm. The fine symmetry of obtained size distribution

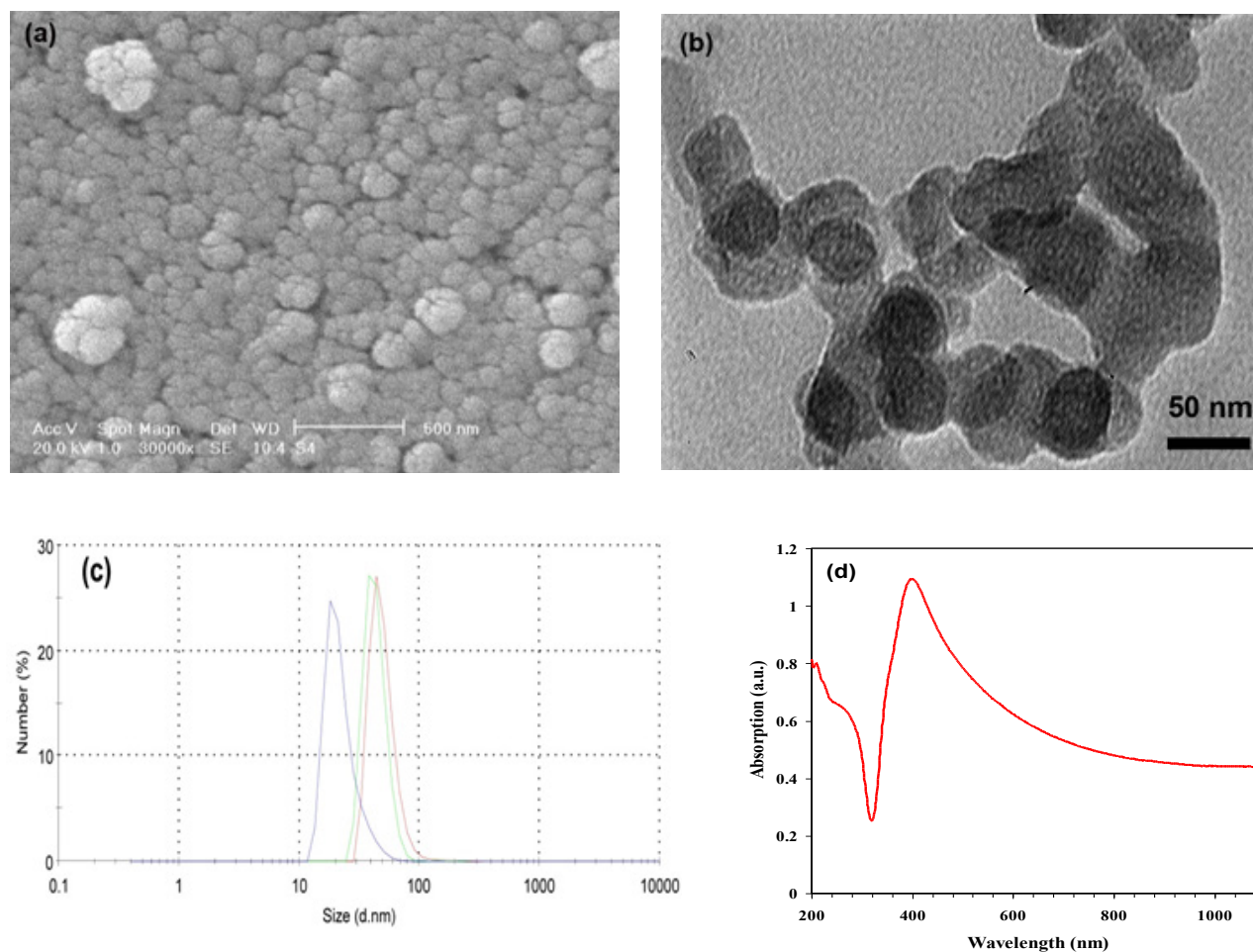


Figure 2. (a) SEM, (b) TEM, (c) DLS and (d) UV-Vis analyzes of prepared silver NPs.

diagrams in DLS data demonstrates uniformity of the formed NPs. In fact, TEM analysis gives a direct size measurement of the particles while DLS analysis gives the size of a hypothetical hard sphere that diffuses in the same fashion as the particle being measured. In other word this is the size of a sphere that has the same translational diffusion coefficient as the particle being measured, assuming a hydration layer surrounding the particle or molecule. Due to the difference in the nature of these two techniques, particle size calculated in DLS average is a relatively bigger than electron microscopy images.

Metallic nanostructures have optical features, which depends on physicochemical properties such as size, shape, concentration, agglomeration state, and refractive index. This makes UV-Vis spectroscopy a valuable technique for identifying, characterizing, and studying nanoscale materials. Figure 2d shows optical absorption spectrum of prepared silver NPs. The absorption peak appeared around 405 nm is the absorbance characteristic of the plasmon of silver NPs and this verifies formation of silver NPs. Therefore, based on the characterizations performed we may conclude that silver NPs with average diameter of 40 nm were formed during the wet chemical method.

The bactericidal activity of the silver NPs were investigated against several gram-negative (*E. coli*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*) and gram-positive (*Staphylococcus aureus*, *Micrococcus luteus* and *Bacillus subtilis*) bacteria, as presented in Figure 3a. It is seen that the number of viable bacteria reduced at the presence of silver NPs for all type of bacteria. This shows that silver NPs have great antibacterial activity which is also well known for decades. However, the efficacy was totally different for different gram-positive and gram-negative bacteria. It was shown that silver must be in its ionized form (Ag^+) to have antibacterial property. In the other hand, gram-positive bacteria have more peptidoglycan than gram-negative bacteria because of their thicker cell walls. Since peptidoglycan is negatively charged and silver ions are positively charged, more silver may be attracted by peptidoglycan in gram-positive bacteria than in gram-negative bacteria leading to more antibacterial efficacy (Ashkarran et al., 2012). One of the possible uses of metallic nanostructures is on environmental applications. Figure 3b shows degradation rate of EY as a model dye in the presence of silver NPs and under UV irradiation using a 125 W high-pressure mercury lamp with 365 nm as strongest wavelength. As it was clear from the optical absorption

spectra, the maximum absorption peak of EY was decreased due to the presence of silver NPs and UV irradiation (dye removal was about 36% after 2 h). This indicates that silver NPs have a weak catalytic activity considering the decomposition rate of the model dye after 2 h.

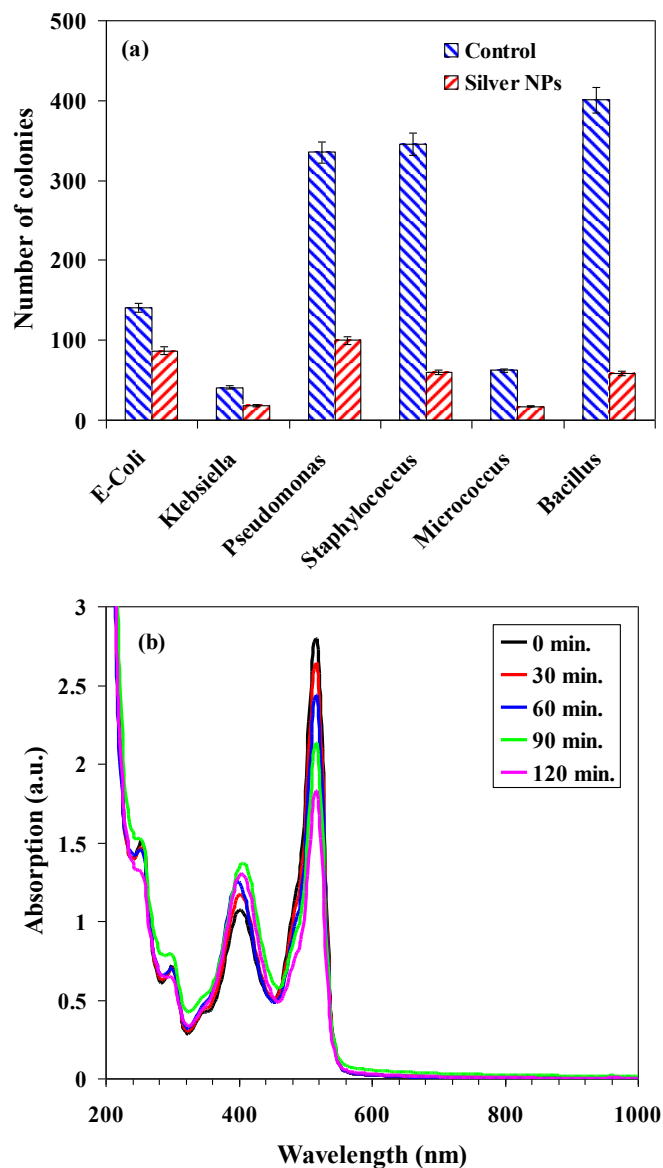


Figure 3. (a) Bactericidal activity of the silver NPs against different gram-negative and gram-positive bacteria and (b) changes in absorption spectrum of EY at the presence of silver NPs.

On the other hand, corona discharge is considered as a fast, efficient and cost effective removal process for treatment of bacterial and environmental contaminations. As we have previously shown that this is a powerful technique for fast decontamination of a wide range of organic compounds (Mohammadi and

Ashkarran, 2016). Herein we applied this technique for degradation of a different model dye (EY). Figure 4 shows the degradation rate of EY at different discharge durations from 1 to 10 min. As it is evident from the Figure 4, the maximum absorption peak of EY (centered at 520 nm) decreased due to the exposure of corona discharge process. This obviously indicates the potential of corona discharge in decomposition of EY dye at ambient environment. Figure 4b illustrates photograph of samples at the beginning and end of discharge process. The untreated EY sample was clear orange while the treated sample shows turned to very pale orange. The calculated dye removal was about 88% after 10 min exposure of corona discharge process. A simple comparison between the degradation rate obtained for EY in the presence of silver NPs and corona discharge reveals that corona discharge is more powerful than silver NPs since the concentration of dye almost reached to zero within 10 min of corona discharge process.

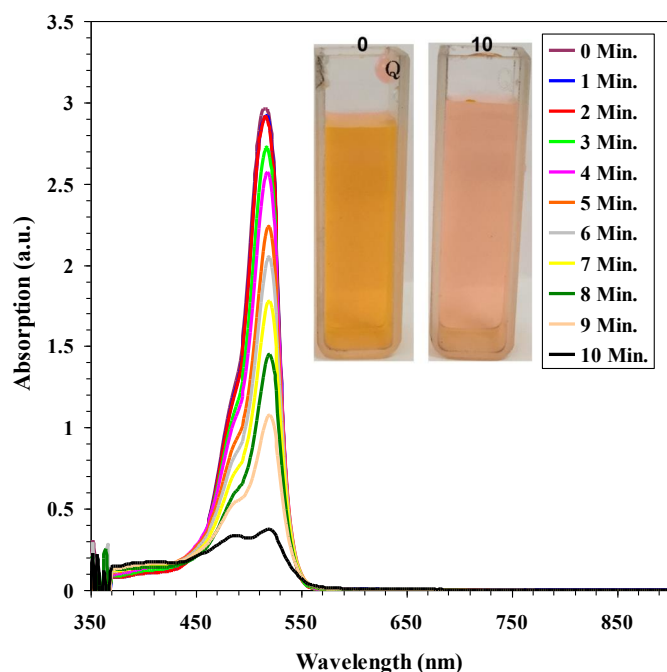


Figure 4. Degradation rate of EY at different discharge durations from 1 to 10 minutes and inset is the corresponding photographs at the initial and final time of process.

Furthermore, the bactericidal ability of corona discharge was investigated against different types of gram-positive and gram-negative bacteria. Figure 5 compare variations of the viable bacteria in the case of gram-positive (*Staphylococcus aureus*, *Micrococcus luteus* and *Bacillus subtilis*) and gram-negative (*E. coli*,

Klebsiella pneumoniae and *Pseudomonas aeruginosa*) bacteria at different exposure times of corona discharge process. It was found that by increasing the discharge time from 1 to 5 min, a remarkable reduction in the number of viable bacteria occurs for all types of bacteria. However, the rate of reduction is almost different from one type of bacteria to another. In fact, the reduction number was more pronounced in gram-positive bacteria than gram-negative. This may due to the negatively charged peptidoglycan component in cell walls of gram-positive bacteria. As we have mentioned before, corona discharge produces energetic positive and negative species. Since positive ions formed during this process are heavier than other negative species such as electrons, they have more contribution in inactivation of bacteria through collision to the peptidoglycan in cell wall (Sari and Fadaee, 2010). The obtained results clearly demonstrated that when compared with other decontaminating techniques such as using silver NPs, antibacterial agents or catalytic materials which has already been published in literatures, corona discharge at ambient air seems to be faster and more efficient (Bauer, et al., 1999; Bhatnagar and Sillanpaa, 2010).

There are different possible mechanisms for growth inhibition of bacteria or degradation of organic dyes under corona discharge process which are considered to be free radicals, charged particles and UV emission from discharge process (Jie et al., 2009). It is very well understood that UV illumination (wavelengths from 100 to 400 nm) has the ability to decompose dye molecules or prevent the growth of bacteria. In fact, UV irradiation can denature the DNA of microorganisms causing death of bacteria or degrade organic molecules such as dyes. In this regard, the bactericidal efficiency and also degradation rate was increased at higher discharge times compared with silver NPs (Bauer et al., 1999). In addition, energetic species available in discharge zone such as atomic oxygen, ozone and also nitrogen oxides may cause antibacterial and degradation properties. Furthermore, formation of energetic free radicals is another possible mechanism which can attack the cell wall of the bacteria and also atomic bindings of dye molecules and make this technique very effective for decontamination purposes.

4. CONCLUSIONS

In summary, we have performed a comparative study of corona discharge and silver NPs for their antibacterial activity towards a series of representative gram-positive and gram-negative bacteria as well as degradation of

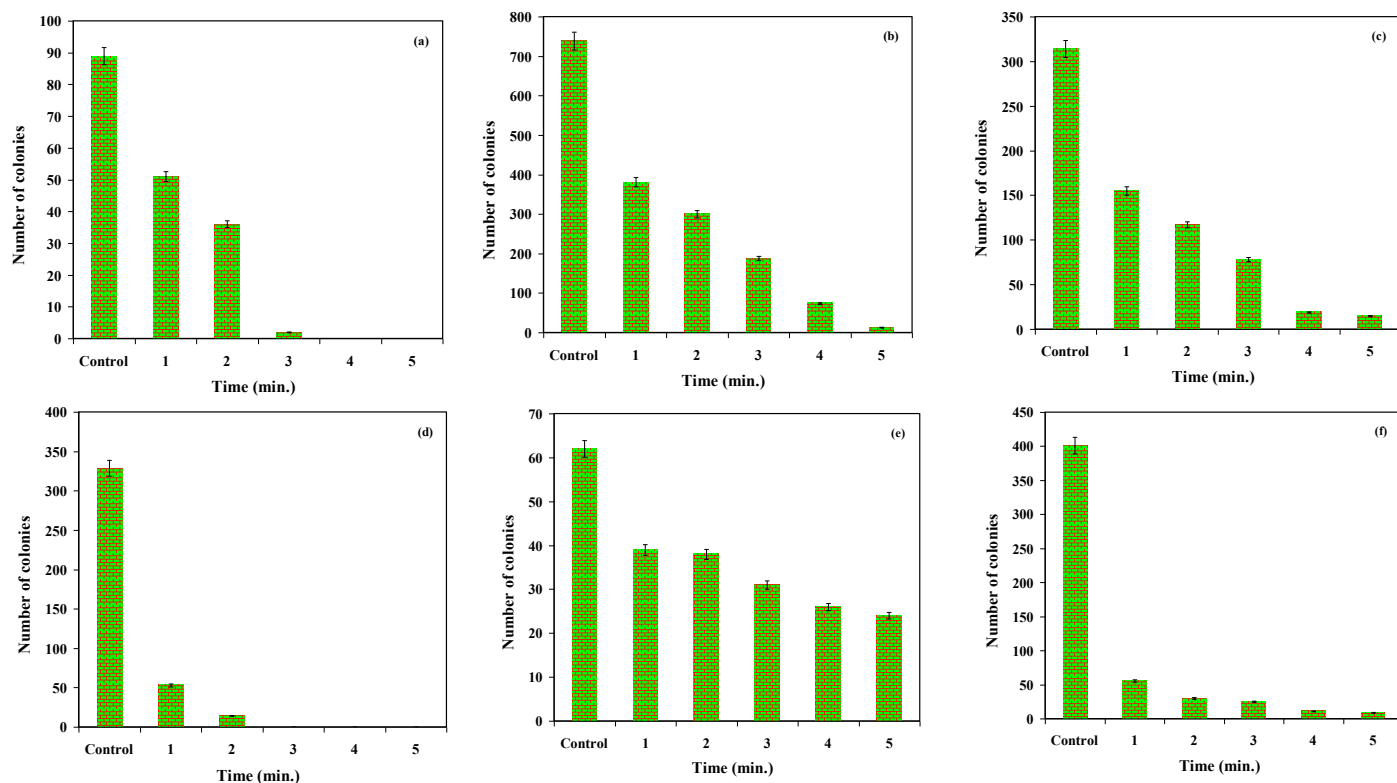


Figure 5. Number of viable bacteria for the gram-negative (a) *E. coli*, (b) *Klebsiella pneumoniae*, (c) *Pseudomonas aeruginosa* and gram-positive (d) *Staphylococcus aureus*, (e) *Micrococcus luteus* and (f) *Bacillus subtilis* bacteria at different exposure times of corona discharge process.

EY dye. We have applied a fast and effective air corona discharge process in ambient air and studied the effect of discharge exposure time on degradation of different bacteria and a model dye. The bactericidal efficacy was however different for various type of bacteria due to their difference in the structure. The maximum inactivation rate was observed for gram-positive bacteria both in the case of silver NPs and corona discharge process. It was suggested that corona discharge is an efficient antibacterial and decontamination process compared to silver NPs as it is able to kill bacteria and decompose organic compounds in a few minutes.

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