FACTORS PROMOTING *STAPHYLOCOCCUS AUERUS* DISINFECTION BY TiO_2, SiO_2 AND AG NANOPARTICLES

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Abstract: The use of conventional disinfection products and methods in drinking water treatment continue to fail in many undeveloped countries. Those applications need to be re-evaluated and innovative approaches to be considered to enhance the reliability and robustness of disinfection while avoiding disinfection byproducts (DBPs) formation. The rapid growth in nanotechnology has prompted significant interest in the environmental applications of nanoparticles (NPs). In order to understand the antibacterial effect of NPs, *Staphylococcus aureus* treated with TiO_2, SiO_2 and Ag NPs were studied under ambient conditions. The results indicated that the most bactericidal effect with specific die-off rates of 0,003 L/mg (TiO_2 NPs) and 0,002 L/mg (Ag NPs) were defined in the absence and the presence of photoactivation, respectively. Moreover, as ionic strength of the test media increases from 10 to 100 mM, NP-NP and NP-bacteria interactions were negatively affected.

Keywords: Disinfection, TiO_2, SiO_2, Ag, Nanoparticles, *Staphylococcus aureus*

Introduction

Among many pathogenic bacteria, Gram (+) *Staphylococcus aureus* are highly infectious and are commonly cause skin, bone and joint infections, and gastrointestinal illness in humans (Lowy 1998, Kadariya 2014). To overcome the potential serious consequences of those infectious pathogens, new technologies and materials for disinfection purposes have been proposed (Hu 2006, Parnia 2009, Oliveira 2014). The conventional disinfectants (i.e. chlorine, ozone, chlorine dioxide and chloramines) known to produce carcinogenic disinfection byproducts (DBPs) (Nieuwenhuijsen 2000, Krasner 2009). Advanced oxidation processes (AOPs) can be applied to reduce the formation of DBPs, to inactivate water pathogens and to mineralize the refractory organic compounds (Chong 2010). Among these AOPs, photocatalytic nanoparticle (TiO_2, ZnO, Fe_2O_3, etc. NPs employing systems have been highly effective on disinfecting the pathogenic bacteria. Matsunaga et al. (1985) reported for the first time that TiO_2 photocatalyst could kill bacterial cells in water by UV illumination, which could generate reactive oxygen species (ROS) in water medium. Since then, numerous photocatalytic disinfection studies under UV light (Wei 1994, Christensen 2003, Gogniat 2006, Bonetta 2013) and fewer studies under solar light (Hu 2007, Helali 2014) using TiO_2 photocatalyst studies have been reported. In addition to those studies, TiO_2 NPs (Shah 2008, Xing 2012, Barnes 2013), TiO_2 thin films (Kambala 2009), Ni doped TiO_2 NPs (Yadav 2014), Fe doped Ti-CNTs (Latif 2014), Ce_2O_3/ TiO_2 composites (Hassan 2012), Ag doped TiO_2 NPs (Kowal 2011), and Al_2O_3-TiO_2-Ag composites (Tartanson 2014) Ag-SiO_2 composite films (Lei 2014), Ag-SiO_2 particles (Sotiriou 2010), and polymer coated Ag NPs (Vukoje 2014), and nano-Ag ions (Feng 2000, Kim 2007, Jung 2008, Sotiriou 2010) were employed to inactivate *S. aureus* and other pathogenic bacteria.

The objective of this study was to evaluate the disinfection efficiency of TiO_2, SiO_2 and Ag NPs on Gram (+) *S. aureus.* Batch experiments were conducted to determine (a) the most antibacterial NP concentrations, (b) the effect of water chemistry on the antibacterial activity of NPs, and (c) the effect of both absence and presence of light on the inactivation of bacteria.

Materials and Methods

Culture of microorganisms: The Gram (+) *Staphylococcus aureus* (ATCC 43300) were cultivated in 100 mL of Luria-Bertani (LB) broth at 37°C on a rotary shaker (150 rpm) for 18 h. The cultures with an initial population of $10^6 \pm 10^2$ CFU mL^{-1} were used in the experiments.

Nanoparticles: Commercially obtained TiO_2, SiO_2 and Ag NPs were used in the experiments (Table 1). A 1000 mg/L stock suspension for each NP was prepared in 0, 10, 50 and 100 mM of deionized water using Na_2HPO_4, KH_2PO_4, NH_4Cl, and NaCl immediately before the experiments. The final concentrations of 10, 100 and 500 mg

L^{-1} NPs were prepared by serially diluting of the stock suspensions having different ionic strength. All experiments were conducted in continuously shaken aqueous slurry solutions to ensure mixing and to prevent settling of the NPs. The bacteria were added to the suspensions immediately prior to the disinfection runs.

Table 1. Nanoparticles used in the experiments.

Nanoparticle	Particle Size (nm)	Surface Area (BET, m^2g^{-1})	Physical appearance
TiO_2 (Anatase)	32	45	White powder
SiO_2	10 - 20	NA	White powder
n-Ag	20 - 40	NA	Black powder

Exposure experiments: To determine the effect of particle concentration and ionic strength of the solutions on the survival of bacteria, dose-response experiments were conducted. Forty five mLs of NP solutions and 5 mL of bacteria prepared in 0, 10, 50 and 100 mM sterile test media were added to each beaker to make initial NP concentrations of 0, 10, 100, and 500 mg L^{-1}. Four artificial light sources (color temperature of 4300 K, and total light intensity of 7000 lux), mimicking the spectrum of natural solar light, were placed 30 cm above the orbital shaker to produce light intensity of *2.1 W* cm^{-2}. The temperature inside the cabinet was maintained at 25.0 ± 1.5◦C. The shaker was set to mix at 150 rpm throughout all experiments. All antibacterial tests were triplicated in presence and absence of light lasted 1 h.

End point test: The numbers of viable cells were determined by LB agar plating. The plates were incubated at 37°C for 24 h, and then the colony counting was done using Lassany model digital colony counter. The survival fractions (N/N_0) and the specific die-off rates (k', Eq 1) (Erdem 2015) were calculated by the following equation:

$$k' \ (L \ \ mg^{-1}) = \frac{-\ln(N/N_0)}{C} \qquad \text{(Eq 1)}$$

where N_0 (CFU, colony forming unit) is the population of the control cultures, N (CFU) is the population of the NP exposed cultures after 1 h in presence and absence of light, and C (mg L^{-1}) is the NP concentration.

Results and Discussion

Nanoparticle characterization:
Particle size, surface area and zeta potential of the NPs were measured NP characterization results are shown in Table 2. Primary particle size results show the measured particle size directly sampled from the box. Nano-TiO_2 primary particle sizes measured by DLS were smaller than the measurement given by the manufacturer, whereas nano-SiO_2 primary particle sizes were bigger than that of the given size. Ag NPs showed an average size value of the primary particle size. It is clearly noted that the water chemistry of the test media affects the NP size. When 10 mM test media was used, the average particle sizes showed a 10 - 15× increase. This result is also confirmed with the SEM images of TiO_2, SiO_2 and Ag NPs that the NP aggregation in test media and NP particle sizes in µm range were observed (Figure 1). The obtained surface areas were inversely correlated to the particle sizes as expected. Zeta potential results showed that the NPs were negatively charged and were not stable in the test media.

Table 2. Characterization results of the TiO_2, SiO_2 and Ag nanoparticles used in the experiments.

	TiO_2	SiO_2	n-Ag
Primary Particle Size by DLS (nm)	26.4	27.7	30.9
Primary Surface Area (BET, m^2g^{-1})	176.34	103.72	92.16
Zeta Potential at pH 6.5 (mV)	-20.3	-17.2	-11.1
Particle Size by DLS (nm) (10 mM, pH 6.5)	287±25	421±19	342±38

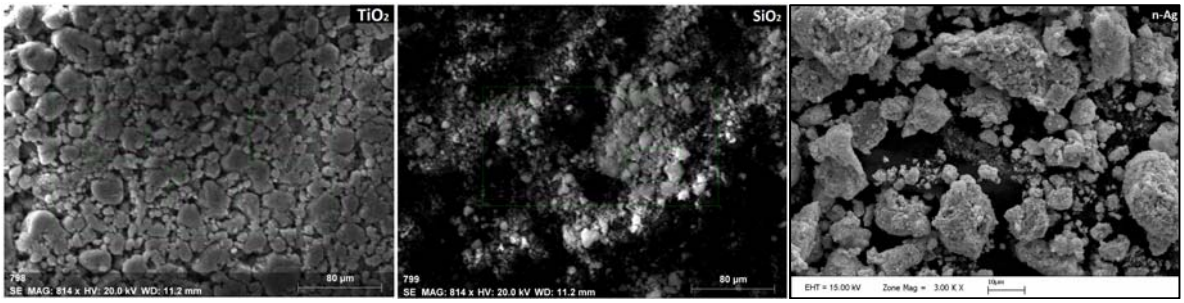

Figure 1. SEM images of TiO_2, SiO_2 and Ag nanoparticles.

Effect of nanoparticle concentration and light:

Four different TiO_2, SiO_2 and Ag NP concentrations (10, 100, 500 and 1000 mgL^{-1}), dark and light (light intensity: *2.1 W cm*$^{-2}$) conditions were used to evaluate the effect of NP concentrations and light on the survival of *S. aureus*. The results given in Table 3 show that regardless of NP type, the mortality rate of the bacteria increased when both NP concentrations increased and changing the light condition from dark to light, especially when 10 mM ionic strength of test media was used. The most effective antibacterial NP concentration in presence of light was confirmed as 1000 mg L^{-1} of TiO_2. In absence of light, when NP concentration increased, the most increase in mortality rates were observed as 57 % (500 mg L^{-1}), 47 % (500 mg L^{-1}) and 40% (1000 mg L^{-1}) in TiO_2, n-Ag and SiO_2 NP setups, respectively. The effects of SiO_2 NPs on bacterial viability were as low as 40 and 56% in absence and presence of light, respectively. TiO_2 NPs were more effective on the *S. aureus* bacteria than n-Ag and SiO_2 NPs in both absence and presence of light.

Table 3. The effect of nanoparticle concentration on the survival of *S. aureus* in absence and presence of light. (Ionic strength: 10 mM, light intensity: *2.1 W cm*$^{-2}$)

NP Concentration (mgL^{-1})	Death (%)					
	TiO_2		SiO_2		Nano-Ag	
	Dark	Light	Dark	Light	Dark	Light
10	13	75	32	33	32	41
100	45	57	27	30	34	49
500	57	76	23	52	47	75
1000	47	93	40	56	32	47

The survival ratios (N/N_0) of the *S. aureus* treated with TiO_2, SiO_2 and Ag NPs under dark (a) and light (b) conditions are depicted in Figure 2. At each concentration of NPs, k' was determined by Eq 1 for both dark/light conditions and ionic strength condition. k' values were averaged (k'_{ave}), where lower k'_{ave} value means higher antibacterial effect. In absence of light, the lowest k'_{ave} values of TiO_2, SiO_2 and n-Ag NPs were calculated as 0.003 L mg^{-1} (0 mM), 0.010 L mg^{-1} (10 mM) and 0.004 L mg^{-1} (50 mM), respectively. The lowest k'_{ave} values of 0.004 L mg^{-1} (50 mM), 0.010 L mg^{-1} (100 mM) and 0.002 L mg^{-1} (10 mM) were calculated when bacteria were exposed to TiO_2, SiO_2 and n-Ag NPs, respectively. The results in Figure 2 show that *S. aureus* was sensitive when lower ionic strength media was used in absence of light. Higher bacterial sensitivity in absence of light may be linked to the release of metal ions or NP-specific mechanisms (Cumberland 2009; Gottschalk 2011; Dobias 2013).

Figure 2. The effect of ionic strength and TiO_2, SiO_2 and Ag nanoparticle concentrations on *S. aureus* under dark (a) and light (b) conditions.

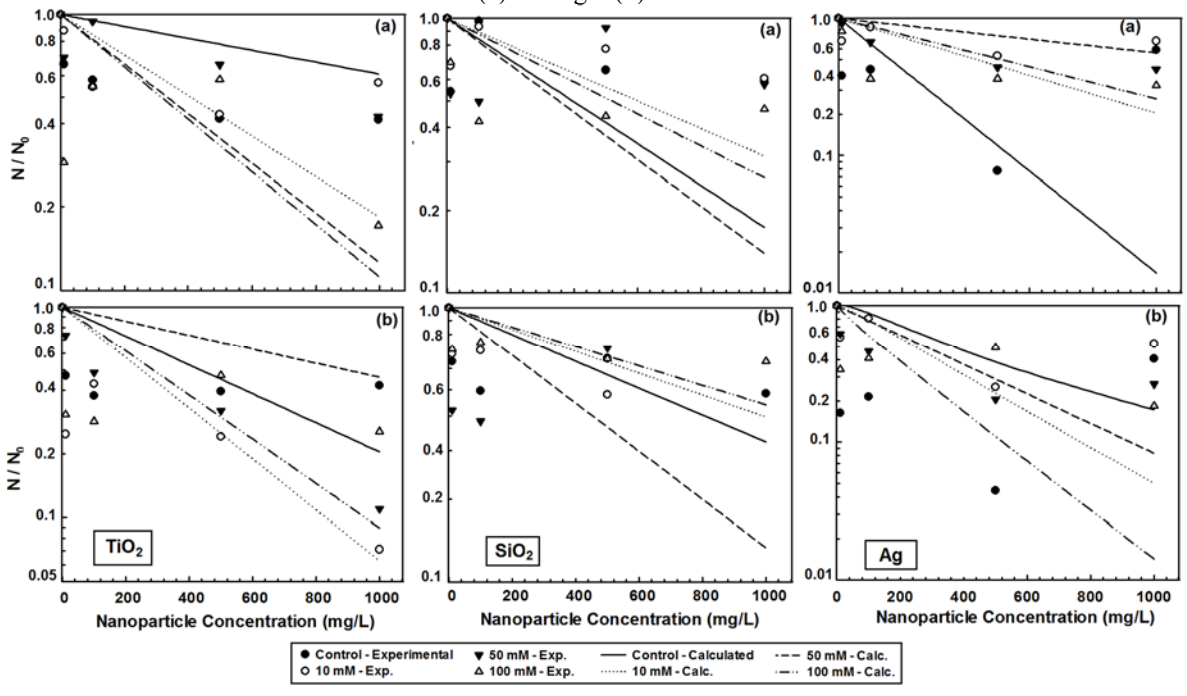

Figure 2 also shows the curve fits obtained from the calculated survival fractions. In order to implement those values, experimentally verified k'_{ave} values were used in Eq 1 and then the calculated survival fractions were estimated. R^2 values were presented in order to correspond the curve fits with experimental results. Highest correspondence ($R^2 = 0.9194$) was observed when *S. aureus* was exposed to TiO_2 NPs under dark, while lowest correspondence ($R^2 = 0.5625$) was observed when SiO_2 NPs were employed. In presence of light, lowest R^2 value of 0.4626 was calculated from bacteria- SiO_2 NPs reactions and highest R^2 value of 0.9563 was observed when *S. aureus* was exposed to nano-Ag particles.

Conclusion

In order to define the factors promoting the disinfection of the *S. aureus* specific die-off rates (k') of the NPs were calculated and were used in this study. It was clearly revealed that TiO_2, SiO_2 and Ag NPs promoted the inactivation of *S. aureus* in both absence and presence of light conditions. The bacteria showed higher sensitivity to TiO_2 and Ag NPs than SiO_2 NPs respectively. The SEM images confirmed that the aggregation/agglomeration of the NPs in test media with different ionic strengths negates the antibacterial activity of the NPs. Therefore, effect of water chemistry on dispersion and retention of NPs needed to be considered. Moreover due to the conduction of the experiments in a relatively clean laboratory conditions, the sustainability of antimicrobial activities of NPs in natural or waste water need to be clarified. Therefore, more research should be conducted to further assess the applicability and sustainability of NPs in disinfection processes.

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