Visible Light Photocatalytic Bacterial Inactivation on Titanium Dioxide Coatings[†]

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Abstract

The increasing number of touch surface mediated infections has steered research to look for alternative strategies that can prevent infection transmission via pathogen inactivation on surfaces. Anatase, a crystalline form of titanium dioxide, shows strong UV induced photocatalytic properties. However, nanoparticles of anatase have been found to inactivate organic contaminants in the visible spectrum. Degradation of mordant orange and inactivation of *S. aureus* was evaluated on anatase surfaces under visible light band pass filters across the visible light region. Inactivation shows a strong co-relation to the absorption spectrum of the dye/microbe on the surface of the anatase coating. The phenomenon is similar to dye sensitized solar cells and was not found to exist in coatings with a higher bandgap such as amorphous silica. Photocatalytic nano-crystalline anatase coatings hold potential as a visible light active disinfectant to inactivate microbes on touch surfaces over long periods of time.

Keywords: TiO₂, visible light photocatalysis, antibacterial coatings

1. Introduction

Decontamination of organic and biological pollutants has gained significant attention owing to the increase in the number of infectious diseases. The increase in the number of hospital acquired infections in the last decade has led to significant research in developing alternate strategies for microbial inactivation and prevention of the transfer of such pathogens.

Hospital acquired infections or nosocomial infections are defined as infections that emanate from a stay in medical facilities and are caused by strains of microbes that have gained resistance to several bactericidal agents (Lobdell et al., 2012). These infections are extremely difficult to treat and have dramatically increased direct costs ranging from \$28 billion to \$45 billion in the United States of America (Scott II, 2009). The number of deaths caused by Methicillin Resistant *Staphylococcus aureus* (MRSA) account to 99,000 annually in the United States

alone (Klein et al., 2007). The transfer of such pathogens causing nosocomial infections is primarily touch surface mediated (Otter et al., 2013; Weber et al., 2013). Thus there is a clear need for inactivation of microbes on these surfaces to reduce the number of nosocomial infections.

Photocatalytic coatings have shown tremendous potential for inactivating organic contaminants and bacteria (Erkan et al., 2006; Lilja et al., 2012; Wei et al., 2014). The principle of photocatalysis for microbial inactivation has been discussed in many reviews and primarily involves the generation of reactive oxygen species (ROS) including hydroxyl free radicals and superoxide free radicals (Fujishima et al., 2008; Henderson, 2011; Linsebigler et al., 1995). Titanium dioxide is one such heterogeneous photocatalyst that is an extremely efficient generator of ROS in the presence of UV light (Ishibashi et al., 2000; Konaka et al., 1999). Inactivation of various bacteria, fungi and oxidative stresses on mammalian cells has been shown due to the ROS generated on UV photo-excited TiO₂ surfaces (Chen et al., 2009; Sunada et al., 2003; Tucci et al., 2013). In addition, complete degradation of organics on TiO₂ surfaces ensures that there is no residue on the surface thereby reducing ‘masking effects’ which often reduce the efficacy of active coatings. Although TiO₂ surfaces show very high potential for photocatalysis, they have only been used for surfaces that can receive a

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significant amount of UV light. Anatase, which is a crystalline form of TiO_2 , has very good photocatalytic properties but absorbs only in the near UV and UV region and has been largely ignored for use in indoor applications. This has led to extensive research into the area of visible light activated photocatalysis on modified titanium dioxide surfaces (Mitoraj et al., 2007; Yu et al., 2003). Doping of TiO_2 with nitrogen and carbon has been shown to reduce the bandgap and thus enhance visible light photocatalysis. Asahi and co-workers have modified titanium dioxide with nitrogen doping to show significant increase in the photocatalytic activity under visible light (Asahi et al., 2001). Shen and group have also shown visible light photocatalysis on anatase surfaces when doped with carbon (Shen et al., 2006). The evaluation of the antibacterial performance of unmodified titanium dioxide in the presence of visible light however has largely been ignored primarily owing to a very small contribution of visible light in anatase induced photocatalysis.

However TiO_2 -based dye sensitized solar cell applications were based on the premise that light-absorbing organic molecules can insert electrons into the TiO_2 conduction band, generate free electrons on TiO_2 and activate photocatalysis (Bach et al., 1998; Krasovec et al., 2009; Oregan and Gratzel, 1991; Yu et al., 2003). We extended this concept using contaminant dyes and microbes to sensitize an otherwise inactive anatase photocatalyst to become active in the visible spectrum (Krishna et al., 2015). This property of anatase can be exploited for coating indoor surfaces which over a long period of time can lead to significant inactivation of microbes and thus reduction in the number of touch surface mediated infections.

2. Experimental section

2.1 Chemical reagents

Most Chemicals were purchased from Fisher Scientific. Mordant Orange was acquired from Sigma-Aldrich Inc. (St. Louis, MO) and anatase and silica were obtained from MKnano (M K Impex Corp. Mississauga, TO).

2.2 Microbial culture and enumeration

Staphylococcus aureus (ATCC 25923) was cultured in Tryptic Soy Agar (Becton, Dickinson and Company, Sparks, MD) and grown for 48 hours in the incubator at 37 °C. An individual colony from the streak plate was recovered and inoculated in Tryptic Soy broth (TSB) and grown to an OD_{600} of 0.6 at 37 °C in a shaker incubator at 150 RPM. The culture was washed thrice and harvested by centrifugation at 4800 RCF and 4 °C. The final concentration for use on the tiles was set to 2×10^6 cells per

ml. Enumeration of bacteria were performed using the pour plate method. 0.1 ml of bacterial suspension was transferred to a petri dish and covered with melted TSA and mixed well. After the agar solidifies at room temperature the plates were incubated at 37 °C for 24 hours.

2.3 Coating preparation

Coatings were made of anatase (7 nm crystallite size) and silica (15 nm crystallite size). The coatings were prepared in accordance with a protocol suggested by Bai et al (Bai et al., 2012). 10 mg of anatase was added to 10 ml of water to have a final concentration of 0.1 wt% and sonicated (Misonix Sonicator 3000, Farmingdale, NY) for 30 minutes at 90 W with NaOH as the electrostatic stabilizer at a pH of around 9.5. The dispersions were then applied as uniform coatings on ceramic tiles of dimensions 2.5 cm $\times$ 2.5 cm, with a total volume of 0.4 ml of coating spread over the surface of the tile. A second coat of anatase was applied after the tiles were dried overnight to ensure complete coverage on the surface of the tile. Coatings of silica were prepared the same way and all coatings were stored in a dark cabinet until use.

2.4 Estimation of bacterial inactivation

Tiles coated with anatase and silica were inoculated with 0.1 ml of bacteria at a starting concentration of $1-2 \times 10^6$ cells per ml. The tiles were then exposed to a light intensity of 1.8 W/m² under fluorescent lamps (General Electric model T8 Ultramax F28T8-SPX41) for 18 h at 23 °C and 40 % RH. A dark control with anatase double coat was also included for the experiments. Microbial recovery from tiles was performed by the sonication of the inoculated tiles at 90 W in 20 ml DI water for 1 minute. 0.1 ml of the bacteria suspension after sonication was enumerated as mentioned before. A zero hour reading taken immediately after initial bacteria inoculation on selected tiles was acquired to estimate the number of bacterial cells that can be recovered from the tile. The tiles were then exposed to visible light under various filters to assess the performance of the coatings at the limits of the absorption spectrum of the contaminant. The recovered microbes were then cultivated in TSA and enumerated using a colony counter. Effect of sonication on bacterial viability was also assessed to eliminate possible inactivation due to sonication

$$\% \text{ Bacterial Inactivation} = \frac{C_0 - C_t}{C_t} * 100 \quad (1)$$

Where C_0 is the concentration of bacteria in CFU at time $t = 0$ and C_t is the concentration of the bacteria at an exposure of time ' t '.

2.5 Estimation of dye degradation

Tiles coated with anatase were inoculated with 0.002 ml of mordant orange at a concentration of 100 ppm. The tiles were then exposed to a light intensity of about $\sim 1.8 \text{ W/m}^2$ for 24, 48, 72 and 96 h at 23 °C and 40 % RH. Reflectance of coated and uncoated tiles were measured with a spectrometer. Absorbance was calculated as the negative $\log_{10}$ of reflectance expressed as a fraction. Dye degradation was calculated by comparing the reflectance measurements of coated tiles at 0 hour exposure to the groups exposed to light.

$$\% \text{ dye degradation} = \frac{A_0 - A_t}{A_t} * 100 \quad (2)$$

Where A_0 is the absorption of dye at time $t = 0$ and A_t is the absorption of the dye at an exposure of time ' t '.

2.6 Effect of optical cut-off filters on the inactivation experiments

Optical filters with cut-off wavelengths were chosen in accordance with the absorption spectrum of the dye and the bacteria. Anatase tiles inoculated with bacteria or dye and were placed under 400, 495 and 550 nm filters. Bacterial and dye degradation was calculated for an exposure of 18 and 24 hours respectively at 23 °C and 40 % RH. The results were then plotted comparing the photocatalytic performance under different cut off filters.

3. Results and discussion

3.1 Absorbance spectrum of mordant orange, anatase and *S. aureus*

The absorbance spectrum of mordant orange, *S. aureus* and anatase on a ceramic tile substrate was acquired using the spectrometer. **Fig. 1** illustrates an absorption cut off for anatase at 380 nm. The absorption of staphylococcus aureus and mordant orange extends well into the visible spectrum with a cut off at around 550 nm.

The spectrum indicates that any absorption in the visible region is accounted for by the dye or the bacterium on the surface of anatase.

3.2 Photocatalytic inactivation of *Staphylococcus aureus*

Photocatalytic inactivation of *S. aureus* was estimated on coatings of anatase and silica. Anatase coating, kept in the dark, was included as a negative control. Test coatings were exposed to a light intensity of about 2 W/m^2 , 32 °C, 40 % RH and an exposure time of 18 h. The spectral dis-

Fig. 1 Absorption spectrum of anatase (green), *S. aureus* (yellow) and Mordant Orange (Orange) across UV and visible spectrum obtained from reflectance measurements on ceramic tiles.

Fig. 2 Spectral distribution of the fluorescent lamp used for bacterial inactivation on tile surfaces. Image courtesy of General Electric Company (General Electric, 2016). Copyright: (2016) G.E.

Fig. 3 Inactivation of *S. aureus* on anatase, silica and in the dark over 18 hours at 23 °C and 40 % RH.

tribution of the fluorescent lamp used for the experiment is shown in **Fig. 2** (GeneralElectric). A 400 nm filter was used for all visible light photocatalysis to eliminate the small amount of UV present in the luminescent bulb spectra. **Fig. 3** shows about 45 % photocatalytic inactivation of *S. aureus* on unmodified anatase surfaces. The in-

activation on silica and dark control are significantly lower and are attributed to desiccation of microbes over the period of 18 h. The experiment indicates a synergy between the bacteria and the anatase surface introducing photocatalysis on anatase surfaces.

The lower inactivation on the silica and dark surfaces also eliminates the contribution of photolysis or oxidative degradation of dye under experimental conditions.

3.3 Contribution of bacteria and dye (contaminant) to the process of visible light photocatalysis

The contribution of contaminants to the process of visible light induced photocatalysis was studied by measuring the inactivation of *S. aureus* as the model bacterium and 5-(4-Nitrophenylazo) salicylic acid, (mordant orange) as a model dye. The kinetics of degradation for mordant orange on unmodified anatase surface were studied as a model for the bacteria. The dye was placed under long pass filters with exposure for 24, 48, 72 and 96 hours. The absorption spectrum of mordant orange exhibits a peak at 410 nm and gradually decreases with a cut off at 550 nm as shown in Fig. 1. Long pass filters cut off wavelengths at 400 nm, 495 nm and 550 nm filters were chosen to ensure non-interference of UV light (400 nm filter), reduced absorbance of dye (495 nm) and cut off of dye absorption (550 nm).

Dye degradation data was analyzed by comparing the reflectance data of dye at the start of the experiment to the tiles at different time points of the experiment. Results in Fig. 4 illustrate a reduction in photocatalytic activity of anatase surfaces with increasing wavelength cut off filters. A sharp decrease in the photocatalytic activity at the 495 filter is seen due to significantly decreased participation of the dye in the process. The photocatalytic degradation under a 550 nm is comparable to the dark control

Fig. 4 Degradation of dye on anatase coatings over a period of 24 h, 48 h, 72 h and 96 h under various optical filters. The absorption of the dye clearly sensitizes the visible light photocatalysis of anatase.

indicating no participation of dye in the process. The reduction in light intensities under various filters were recorded and show a maximum reduction of 5 % at the 495 nm cut off and 16 % at the 550 nm cutoff wavelengths. Subsequently, inactivation of *S. aureus* on anatase surfaces was carried out under similar spectral conditions to validate the mechanism of photocatalytic inactivation with biological contaminants. The results plotted in Fig. 5 show that *S. aureus* inactivation under visible light gradually decreased with increasing cut off limits up to 550 nm in accordance with dye degradation results. It is also noted from the absorption spectrum in Fig. 1 that the only component absorbing in the visible spectrum is the bacteria. Additionally, the trend of bacterial inactivation on anatase in visible light follows the trend in absorption intensity of bacteria. This indicates the antibacterial potential of unmodified anatase with visible light.

From the data obtained we hypothesize that the mechanism of microbial inactivation on unmodified anatase surfaces is similar to that of TiO_2 dye sensitized solar cells (DSSC). In TiO_2 based DSSC's irradiation with visible light leads to (i) excitation of the dye molecule, (ii) subsequent oxidation of dye molecule, and (iii) transfer of electrons from the dye molecule to the TiO_2 conduction band. The electrons then diffuse through the circuit to the counter electrode. The electrolyte is reduced from I_3^- to I^- which diffuses and combines with the oxidized dye molecule to complete the circuit. Similarly in a contaminant activated photocatalyst, the dye or microbe acts as the electron source when irradiated with visible light. Fig. 6 is a schematic of the hypothesized mechanism of action on unmodified anatase surfaces when in contact with or-

Fig. 5 Inactivation of *S. aureus* on anatase under different optical filters over 18 hours at 23 °C and 40 % RH. The inactivation of bacteria decreases with the increase in cut off wavelengths of the long pass filters from 400 to 550 nm.

Fig. 6 Schematic for possible mechanism of inactivation on unmodified TiO_2 surfaces (Krishna et al., 2016).

ganic contaminants such as microbes (Krishna et al., 2015). Electrons inserted in the conduction band of TiO_2 catalyze the small molecules like oxygen and water into super oxide free radicals. These reactive radicals in turn oxidize most organic molecules to CO_2 and water. The generation of free radicals in the photocatalyst with availability of microbial contaminants leads to their continued inactivation.

4. Conclusions

The present study illustrates the potential of unmodified nano-crystalline anatase as a photocatalytic disinfectant in visible light when in contact with microbes. While the kinetics of inactivation of bacteria on such unmodified surfaces is not as fast as in the case of conventional disinfectants, photocatalytic coatings can last much longer and have a very high cumulative contribution towards surface disinfection. Masking by inactivated microbes often hampers the performance of active coatings by forming a barrier between the active species and the microbes. However, the complete degradation of microbes (organic contaminants) on anatase surfaces ensures no ‘masking effect’ unlike on other potent antimicrobial surfaces like copper and silver. Thus, anatase surfaces in comparison can function for long inactivation periods. We would also like to further indicate that these coatings can be applied to surfaces and complemented with additional disinfection treatments to have increased efficacies of surface disinfection. A combination of disinfection approaches can be used for surfaces that do not receive large amounts of UV light and kill pathogens that are transferred through touch mediation.

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Nomenclatures

CFU/ml	Colony forming units per ml
A_0	Absorbance at time 0
A_t	Absorbance at time t
C_0	Concentration at time 0 (CFU/ml)
C_t	Concentration at time t (CFU/ml)
RCF	Relative Centrifugal Force
RH	Relative Humidity (%)
RPM	Rotations per minute
W/m^2	Watt/ Square meter

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