Journal of Hazardous Materials 165 (2009) 506–510

Contents lists available at ScienceDirect

Journal of Hazardous Materials

journal homepage: www.elsevier.com/locate/jhazmat

# Photocatalytic inactivation of *Bacillus anthracis* by titania nanomaterials

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ARTICLE INFO

*Article history:*
Received 28 June 2008
Received in revised form
21 September 2008
Accepted 6 October 2008
Available online 14 October 2008

*Keywords:*
Photocatalysis
*Bacillus anthracis*
Nanosized titania
Inactivation

ABSTRACT

Photocatalytic inactivation of *Bacillus anthracis* was studied by using titania nanomaterials and UVA light. Experimental data clearly indicated that, time of exposure, quantity of catalyst, intensity of light, particle size and Sunlight affected the inactivation. It also demonstrated the pseudo-first order behavior of inactivation kinetics and pointed out the enhanced rate of inactivation in the presence of nano-titania existing as a mixture of anatase and rutile phases. The values of rate constant were found to increase when the quantity of catalyst and intensity of UVA light were increased. Nanosized titania exhibited better inactivation properties than the bulk sized titania materials. Sunlight in the presence of nano-titania (mixture of anatase and rutile phases) displayed better photocatalytic bactericidal activity of *B. anthracis* than sole treatment of Sunlight.



## 1. Introduction

*Bacillus anthracis*, one of the most pathogenic microorganisms and etiological agent of deadly disease anthrax has attracted much attention and been in the lime light due to its significance in public health and military sectors [1]. It occurs in three forms, namely cutaneous, gastrointestinal and pulmonary and causes distinct clinical symptoms like flu followed by respiratory collapse, serious gastrointestinal difficulty, vomiting of blood, severe diarrhoea, boil-like skin lesions, etc. [2,3]. Owing to these highly lethal properties, it has been designated as biological warfare (BW) agent and more recently, its spores were used for bioterrorism in 2001 after the world trade centre attack in United States. Inactivation or decontamination of such agent is one of the most challenging tasks in relation to public health and environmental safety.

Many technologies have been employed till date for the decontamination of water polluted with similar kind of microorganisms. Activated carbon filtration, chlorination, ozonation, reverse osmosis have been used for the decontamination purposes, however, certain disadvantages like disposal problems, production of toxic by-products like trihalomethanes, high cost, bacteria fouling, etc., prompted scientists to search for alternative technologies [4–10]. Recently, direct illumination of contaminated water with UV light centered at 254 nm has been established as a possible method for photolytic disinfection or decontamination at room temperature. However, this type of radiation is injurious to health and involves occupational risks [11–15]. Thereafter, this photolytic disinfection method has been modified as photocatalytic oxidation (PCO) method by integrating semiconductor materials like titania along with UVA light (320–400 nm). This PCO method displays excellent disinfection efficiency towards microorganisms avoiding most of the above disadvantages of other methods, hence, has been widely used. In this regard, several bacteria like *Escherichia coli*, *Lactobacillus helveticus*, *Salmonella choleraesuis*, *V. parahaemolyticus*, *L. monocytogenes*, etc. have been successfully decontaminated by using the synergistic photolytic and PCO methods facilitated by bulk sized titania particles and UVA light [15–20]. In addition to these, photocatalytic properties of titania particles were critically influenced by the size of the particles, phase of titania, i.e., anatase or rutile, concentration of catalyst, irradiation time, intensity of radiation, etc. [21,22].

Besides these, several methods for isolation and identification of *B. anthracis* have been reported [23] until today, however, nothing was reported on the inactivation of this microorganism by nana titania. Inspired by the above studies, we have made an attempt to study the photolytic and photocatalytic inactivation of non-pathogenic *B. anthracis* by using the synchronized effect produced by UVA light and titania nanoparticles in anatase and rutile phases and compared the data with that of bulk titania.

## 2. Experimental

### 2.1. Materials

Non-pathogenic *B. anthracis* Sterne strain was obtained from the Institute of Veterinary and Preventive Medicine, Ranipet, Vellore. It

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0304-3894/$ – see front matter © 2008 Published by Elsevier B.V.
doi:10.1016/j.jhazmat.2008.10.009

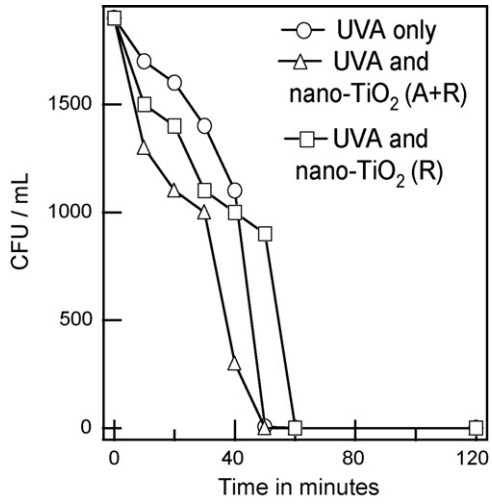


**Fig. 1.** Photocatalytic inactivation of *Bacillus anthracis* under the treatment of only UVA light and UVA light in the presence of nano-$TiO_2$ (A + R) or nano-$TiO_2$ (R) as a function of time at 1.2585 mW/cm$^2$, using 25 mg of catalyst.

was grown in a 250 mL Erlenmeyer flask containing nutrient broth in a shaking incubator at 37 °C for over night. The biomass was diluted with phosphate buffer saline at pH 7.2 and cell counts were measured with turbidity readings at 600 nm with spectrophotometer (Molecular Devices, USA) and the cell counts were correlated with plate count.

Nanosized $TiO_2$ samples nano-$TiO_2$ (A + R) (13463-67-7, mixture of anatase and rutile phases, average size ~70 nm) and nano-$TiO_2$ (R) (1317-80-2, only rutile phase, average size ~40 nm) were obtained from Sigma–Aldrich chemicals, USA. Bulk sized $TiO_2$ (1317-80-2, average size 1–2 μm) was also procured from Sigma–Aldrich chemicals, USA.

### 2.2. Photocatalysis and analysis

Aqueous solutions containing ~700–1900 CFU/mL of *B. anthracis* were magnetically stirred with and without nanosized and bulk sized titanium dioxide at room temperature (30 ± 1 °C) under the illumination of UVA light (320–400 nm) for investigating the photolytic (without photocatalyst) and photocatalytic inactivation of *B. anthracis*. The 100 μL of samples were withdrawn at regular intervals of time for plate count experiments. Subsequently, the plates were incubated at 37 °C and colony counts were taken after 24 h. UVA light irradiation experiments were performed in a photoreactor obtained from M/s. Luzchem, Canada of LZC 4V model. The intensity of light was varied by glowing more number of lights. Intensity of light was measured by digital light meter (SLM 110 model) of A.W. Sperry Instruments, USA with the help of the adopters provided.

## 3. Results and discussion

The synergistic effect of UVA light treatment and the addition of nanosized titania particles on the inactivation of *B. anthracis* was studied by using UVA light of intensity 1.2585 mW/cm$^2$ and by adding 75 mg of either nano-$TiO_2$ (A + R) or nano-$TiO_2$ (R) to *B. anthracis* contaminated water. Plate counts were recorded at various intervals of time until 60 min and the data is illustrated in Fig. 1. As per the figure, 100% of *B. anthracis* (1900 CFU/mL) was observed to be inactivated within 50 min due to the combined treatment of nano-$TiO_2$ (A + R) (sample existing as a mixture of anatase and rutile phases) and UVA light. Only 63% of *B. anthracis* was seemed to be inactivated within 50 min when the contaminated water was treated with UVA light. This observation can be attributed to the additive effect of nano-titania existing as a mixture of anatase and rutile phases. However, only 52% *B. anthracis* was seemed to be inactivated when the contaminated water was treated with nano-$TiO_2$ (R) (nano-titania existing as only rutile phase) along with UVA light indicating the absence of noticeable additive effect thereby illustrating inferior photocatalytic properties of rutile phase titania. In addition to these, Fig. 1 also displayed the exponential decrease of the remaining *B. anthracis* (CFU) with the progression of time exemplifying the pseudo-first order kinetics. As can be seen in Fig. 2, the plot of Log (CFU/mL) on *Y*-axis and time on *X*-axis illustrated linear curves for the above three types of treatments confirming pseudo-first order behavior of photolytic and photocatalytic inactivation of *B. anthracis*. The values of kinetics rate constant and half-life of inactivation were established from the slope (slope × 2.303, 0.6932/k) of the linear curves and were found to be 0.010 min$^{-1}$ and 69.32 min when solely UVA light was used. Figs. 1 and 2 also indicate the low kinetics rate at initial stages (0–30 min) (half-life 69.32 min) and enhanced rate at later stages (30–60 min) (half-life 21.7 min) of inactivation. This observation can be ascribed to drastic enhancement of rate of inactivation on prolonged exposure to UVA light and is consistent with the reported data [24]. The values of kinetics rate constant and half-life were observed to be 0.040 min$^{-1}$, 0.010 min$^{-1}$ and 17.33 min, 43.3 min, respectively for UVA light in the presence of nano-$TiO_2$ (A + R) and nano-$TiO_2$ (R). Comparative examination of data clearly indicates that, *initial* rate of inactivation of *B. anthracis* is strongly enhanced (four times) by the addition of nano-$TiO_2$ (A + R) to UVA light and 1.6 times when nano-$TiO_2$ (R) was added to UVA light. Although, UVA light photolytically inactivate the bacteria by inducing DNA lesions due to the formation of dimeric pyrimidine photoproducts, its effect is seemed to get enhanced by the presence of nanosized titania [21,22,25]. Titanium dioxide in the anatase form inherently behaves as semiconductor and upon illumination of $TiO_2$ dispersed in water with UVA light, excess electrons are generated in the conduction band and positive holes are generated in the valance band. Subsequently, the photon generated electrons and holes migrate to the surface of titania and serve for redox reactions. On the surface of titania the photo generated hole sites in the valance band of titania seemed to react with water or surface hydroxide groups to form hydroxyl radicals (OH•), whereas excess electrons promoted to the conduction band seemed to react with molecular oxygen to

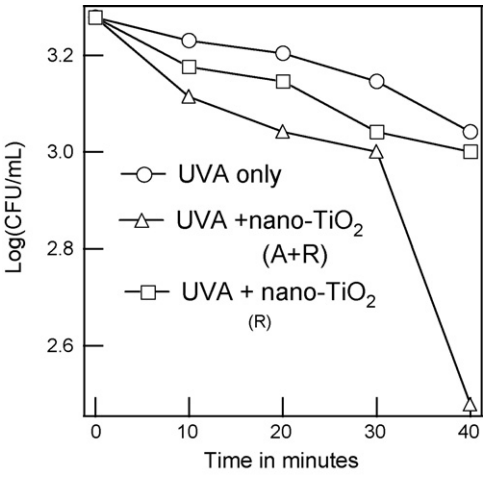


**Fig. 2.** Kinetics of photocatalytic inactivation of *B. anthracis* under the treatment of only UVA light and UVA light along with nano-$TiO_2$ (A + R) or nano-$TiO_2$ (R) as a function of time at 1.2585 mW/cm$^2$ using 25 mg of catalyst.

**Table 1**
Effect of catalyst quantity (in mg) on kinetics of inactivation of *Bacillus anthracis* of 1900 CFU/mL at 1.2585 mW/cm$^2$.

| Amount of catalyst (mg) | Only UVA rate ($min^{-1}$) | Only UVA half-life (min) | UVA + $TiO_2$ (A + R) rate ($min^{-1}$) | UVA + $TiO_2$ (A + R) half-life (min) | UVA + $TiO_2$ (R) rate ($min^{-1}$) | UVA + $TiO_2$ (R) half-life (min) |
|---|---|---|---|---|---|---|
| 10 | 0.010 | 69.32 | 0.010 | 69.32 | 0.007 | 99 |
| 25 | 0.010 | 69.32 | 0.010 | 69.32 | 0.007 | 99 |
| 50 | 0.010 | 69.32 | 0.040 | 17.33 | 0.008 | 86.65 |
| 75 | 0.010 | 69.32 | 0.040 | 17.33 | 0.016 | 43.32 |
| 100 | 0.010 | 69.32 | 0.060 | 11.55 | 0.050 | 13.86 |

**Table 2**
Effect of intensity on kinetics of inactivation of *B. anthracis* of 1900 CFU/mL using 25 mg catalyst.

| Intensity of UVA in mW/cm$^2$ | Only UVA rate ($min^{-1}$) | Only UVA half-life (min) | UVA + $TiO_2$ (A + R) rate ($min^{-1}$) | UVA + $TiO_2$ (A + R) half-life (min) | UVA + $TiO_2$ (R) rate ($min^{-1}$) | UVA + $TiO_2$ (R) half-life (min) |
|---|---|---|---|---|---|---|
| 0.4195 | 0.010 | 69.32 | 0.010 | 69.32 | 0.008 | 86.65 |
| 0.8390 | 0.010 | 69.32 | 0.010 | 69.32 | 0.007 | 99 |
| 1.2585 | 0.010 | 69.32 | 0.010 | 69.32 | 0.007 | 99 |
| 1.5941 | 0.020 | 34.66 | 0.030 | 23.10 | 0.020 | 34.66 |
| 2.4331 | 0.055 | 12.60 | 0.035 | 19.80 | 0.010 | 69.32 |

form superoxide ions which further react with water to form additional amount of hydroxyl radicals [15–20]. These $OH^{\bullet}$ seemed to be responsible for the above synergistic inactivation of *B. anthracis* in the presence of titania nanoparticles by photocatalytic oxidation method.

Afterwards, the effect of quantity of catalyst on photocatalytic inactivation of *B. anthracis* was investigated by varying the quantity of nanosized titania either nano-$TiO_2$ (A + R) or nano-$TiO_2$ (R) while keeping the concentration of bacteria (~1900 CFU/mL) and intensity (1.2585 mW/cm$^2$) of UVA light constant and the results are incorporated in Table 1. As per Table 1, when the amount of nano-$TiO_2$ (A + R) was increased from 10 mg to 100 mg, the value of kinetics rate constant of inactivation of *B. anthracis* was found to increase from 0.010 $min^{-1}$ to 0.060 $min^{-1}$ while the value of half-life decreased from 69.32 min to 11.55 min. In the case of nano-$TiO_2$ (R) the value of kinetics rate constant was found to increase from 0.007 $min^{-1}$ to 0.050 $min^{-1}$ while the value of half-life decreased from 99 min to 13.86 min when the quantity of the catalyst was increased from 10 mg to 100 mg. Whereas, the values of kinetics rate constant and half-life under sole treatment of UVA light were found to be 0.010 $min^{-1}$ and 69.32 min. The data also indicate that nanosized titania containing mixture of anatase and rutile crystalline phases exhibited considerably enhanced photocatalytic activity than that observed due to sole treatment with UVA light or that with nano-$TiO_2$ (R) and this observation can be attributed to the presence of anatase phase in the former material. Anatase phase of titania possesses better photoactivity when compared to rutile phase due to the larger Fermi level in the former as per the previously reported literature [26].

Thereafter, effect of intensity of UVA light on photolytic and photocatalytic inactivation of *B. anthracis* was explored and the data is included in Table 2. This table demonstrates that, when the intensity of light was increased from 0.4195 mW/cm$^2$ to 1.5941 mW/cm$^2$, the value of rate constant of inactivation under the sole treatment of UVA light seemed to increase from 0.010 $min^{-1}$ to 0.020 $min^{-1}$ while the value of half-life decreased from 69.32 min to 34.66 min. The value of rate constant in the case of nano-$TiO_2$ (A + R) increased from 0.010 $min^{-1}$ to 0.030 $min^{-1}$ when the intensity was varied from 0.4195 mW/cm$^2$ to 1.5941 mW/cm$^2$. Whereas, in the case of nano-$TiO_2$ (R) when the intensity was increased from 0.4195 mW/cm$^2$ to 1.5941 mW/cm$^2$, the value of rate constant was found to increase from 0.008 $min^{-1}$ to 0.020 $min^{-1}$. Analysis and comparison of kinetic data indicate the enhanced photocatalytic activity of titania containing the mixture of anatase and rutile phases, however, rutile phase exhibited even poorer activity relative to the sole treatment of UVA in the above mentioned range of intensity. However, when the intensity was increased up to 2.4331 mW/cm$^2$ the UVA effect surpassed the additive effect of titania as per the data observed in Table 2. It also reveals that the rate of inactivation of *B. anthracis* considerably increased with the increase of intensity of UVA light.

In addition to this, effect of particle size of titania on the photocatalytic inactivation of *B. anthracis* was studied and the data is shown in Table 3. As per the data portrayed in Table 3, the values of kinetics rate constant and half-life of inactivation in the case of UVA and nano-$TiO_2$ (A + R) (particle size ~70 nm) were observed to 0.01 $min^{-1}$ and 69.32 min while for UVA and bulk $TiO_2$ (A + R) (particle size ~1–2 μm) they were observed to be 0.006 $min^{-1}$ and 115.5 min. In the case of UVA light and nanosized $TiO_2$ (R) (particle size ~40 nm), the values of kinetics rate constant and half-life were observed to be 0.007 $min^{-1}$ and 99 min while bulk $TiO_2$ (R) (particle size ~1–2 μm) did not participate in inactivation at all. As indicated by the above data, relatively nano-$TiO_2$ (A + R) or nanosized $TiO_2$ (R) exhibited better inactivation efficiencies than bulk $TiO_2$ (A + R) or bulk $TiO_2$ (R). This observation can be accredited to larger specific surface area, larger number surface active sites and enhanced surface charge carrier transfer rate of nanosized $TiO_2$ samples like nano-$TiO_2$ (A + R) (50 m$^2$/g) or nanosized $TiO_2$ (R) (190 m$^2$/g) relative to bulk samples [surface area of bulk $TiO_2$ (A + R) is 6 m$^2$/g and surface area of bulk $TiO^2$ (R) is 5 m$^2$/g].

After this, we have investigated the combined effect of Sunlight and nano-$TiO_2$ (A + R) or nano-$TiO_2$ (R) on the inactivation of

**Table 3**
Effect of particle size of titania on photocatalytic inactivation of *B. anthracis* at 1.2585 mW/cm$^2$ using 25 mg of catalyst.

| Source of inactivation | UVA + $TiO_2$ (A + R) | | UVA + $TiO_2$ (R) | |
|---|---|---|---|---|
| | Average size | Rate (half-life) $min^{-1}$ (min) | Average size | Rate (half-life) $min^{-1}$ (min) |
| Nanoparticles | ~70 nm | 0.01 (69.32) | ~40 nm | 0.007 (99) |
| Bulk particles | ~1–2 μm | 0.006 (115.5) | ~1–2 μm | No inactivation |

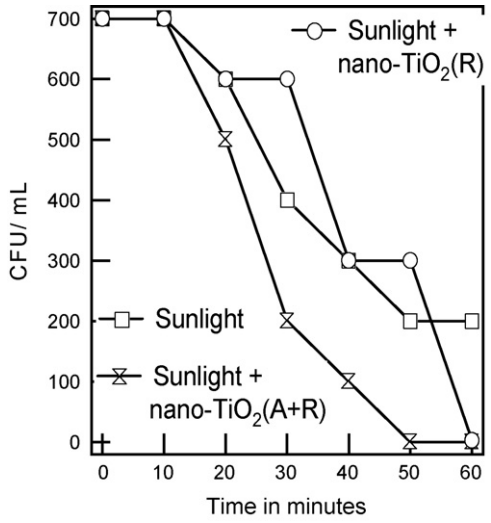


**Fig. 3.** Effect of Sunlight on the photocatalytic inactivation of ~700 CFU/mL of *B. anthracis* in the presence of nano-$TiO_2$ (A + R) or nano-$TiO_2$ (R) as a function of time by using 25 mg of catalyst.

non-pathogenic *B. anthracis* and the graph is portrayed in Fig. 3. It indicates 71%, 100% and 57% of inactivation of *B. anthracis* in 60 min, 50 min and 60 min under the sole exposure of Sunlight and that in the presence of nano-$TiO_2$ (A + R) or nano-$TiO_2$ (R), respectively. Apparently, the addition of nano-$TiO_2$ (A + R) augmented the inactivation efficiency of bacteria indicating the synergism in activities occurred due to Sunlight and nano-$TiO_2$ (A + R). As we know, Sunlight inactivates the microorganisms due to the synchronized effect of UV and IR parts of it and his power varies with time in a day and also when the clouds are passing by. In the present case we have performed our experiments at around 11:00 a.m. (Indian standard time) and we have not measured the intensity of Sunlight. Later the graphical plot was made by taking Log (CFU/mL) on *Y*-axis and time on *X*-axis and it shows the linear curves indicating the pseudo-first order behavior of the inactivation kinetics of *B. anthracis* either in the presence of nanosized titania particles or under the sole treatment of Sunlight. The values of kinetics rate constant and half-life were observed to be 0.020 $min^{-1}$ and 30.1 min, 0.050 $min^{-1}$ and 13.8 min, 0.020 $min^{-1}$ and 34.6 min, respectively for only Sunlight, Sunlight in the presence of nano-$TiO_2$ (A + R), Sunlight in the presence of nano-$TiO_2$ (R). The data clearly indicates the occurrence of enhanced inactivation of *B. anthracis* due to the addition of nano-$TiO_2$ (A + R), while, nano-$TiO_2$ (R) exhibited poorer inactivation efficiency and is consistent with the above observations. All the above observations collectively illustrated the superior competence of photocatalytic oxidation method facilitated by using titania nanomaterials and UVA light or Sunlight in the decontamination of deadly bacteria *B. anthracis*. This method uses UVA light that is relatively less hazardous than UVC light which is very injurious to health. It also uses minimum amount of titania (as a mixture of anatase and rutile) due to the advantage of nano-size over the bulk sized titania. The nano-sized titania possesses superior photocatalytic properties over bulk sized owing to high surface-to-volume ratio. This method due to established bactericidal mechanisms have not only improved the safety level by reducing the microorganism re-growth but also justified the complete decontamination of *B. anthracis*. Moreover, it has the advantages such as low cost, absence of disposal problems, bacterial fouling and toxic by-product formation which are expected to occur in the case of the other methods thereby promising futuristic biological warfare decontamination applications.

## 4. Conclusions

The above observations clearly indicate that photolytic and photocatalytic inactivation of non-pathogenic *B. anthracis* assisted by titania nanomaterials and UVA light. Exposure for longer intervals of time enhanced the percentage of inactivation of *B. anthracis*. Increase in the quantity of catalyst, intensity of UVA light enhanced the rate of inactivation of bacteria. Nanosized titania exhibited better photocatalytic bactericidal properties than bulk material and Sunlight in the presence of nano $TiO_2$ (mixture of anatase and rutile) exhibited dominating photoactivity than sole treatment or that in the presence of rutile phase.

## Acknowledgement

Authors thank Aravind Tiwari for extending timely support.

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