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Investigation of UV–TiO_2 photocatalysis and its mechanism in *Bacillus subtilis* spore inactivation

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ABSTRACT

The inactivation levels of *Bacillus subtilis* spores for various disinfection processes (ultraviolet (UV), TiO_2 and UV–TiO_2) were compared. The results showed that the inactivation effect of *B. subtilis* spores by UV treatment alone was far below that for bacteria without endospores. TiO_2 alone in the dark, as a control experiment, exhibited almost no inactivation effect. Compared with UV treatment alone, the inactivation effect increased significantly with the addition of TiO_2. Increases of the UV irradiance and TiO_2 concentration both contributed to the increase of the inactivation effect. Lipid peroxidation was found to be the underlying mechanism of inactivation. Malondialdehyde (MDA), the degradation product of lipid peroxidation, was used as an index to determine the extent of the reaction. The MDA concentration surged surprisingly to 3.24 nmol/mg dry cell with the combination disinfection for 600 sec (0.10 mW/cm^2 irradiance and 10 mg/L TiO_2). In contrast, for UV alone or TiO_2 in the dark, the MDA concentration was 0.38 and 0.25 nmol/mg dry cell, respectively, under the same conditions. This indicated that both UV and TiO_2 were essential for lipid peroxidation. Changes in cell ultrastructure were observed by transmission electron microscopy. The cell membrane was heavily damaged and cellular contents were completely lysed with the UV–TiO_2 process, suggesting that lipid peroxidation was the root of the enhancement in inactivation efficiency.
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Introduction

Ultraviolet (UV) disinfection technology, with the advantage of high efficiency and operational convenience, has the potential to replace the traditional chlorine disinfection process (Bolton and Linden, 2003). However, due to the limitations of physical disinfection, other chemicals are required to maintain the capability of continuous disinfection and to improve the inactivation level (Gao et al., 2009; Matafonova and Batoev, 2012; Sun et al., 2013). Advanced oxidation processes (AOPs), especially UV-based processes such as UV–H_2O_2 (Shu et al., 2013), UV–Cl_2 (Wang et al., 2011), UV–O_3 (P. Liu et al., 2010) and UV–TiO_2 (Qi et al., 2007), are able to produce highly reactive radicals by photolysis. UV–TiO_2 technology was reported for the first time by Matsunaga et al. (1985), who found that by contacting them with TiO_2/Pt particles under UV exposure for 60 to 120 min, microbial cells could be completely sterilized. Since then, it has attracted ever-increasing intensive research interest in compound degradation (Kent et al., 2011; Shigwedha et al., 2007).

Nevertheless, whether UV–TiO_2 is capable of inactivating microorganisms in water is still being studied. Benabbou et al. (2007) confirmed that the combination of UV and TiO_2 was a

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promising alternative for *Escherischia coli* inactivation. Cho et al. (2005) found that the addition of TiO_2 contributed to UV treatment in MS-2 phage inactivation efficiency. Despite the many investigations on the inactivation behaviors of UV–TiO_2, the understanding of its photochemical mechanism is not complete. According to previous papers, there have been a number of inactivation mechanism explanations advanced for the UV-based AOP, for example, the inhibition of DNA replication (Yakobson et al., 1989), the alteration of membrane structure (X. Liu et al., 2010), the restriction of catalase activity (Zigman et al., 1996), and lipid peroxidation (Sokmen et al., 2001).

Lipid peroxidation, the oxidative degradation of polyunsaturated fatty acids, has been extensively studied. It is attributed to the oxidation of cell membrane lipids by free radicals, leading to membrane destruction (Marnett, 1999). The process gives rise to inevitable cell lethality, since cell function depends heavily on an intact membrane structure (Maness et al., 1999). As a result, lipid peroxidation has been proposed as the most hazardous effect on cells by free radicals (Miura et al., 2002). Nevertheless, few papers have recognized the relationship between lipid peroxidation and UV–TiO_2 technology, especially for *Bacillus subtilis* spore inactivation. As a result, this paper focused on investigating the level of lipid peroxidation, further complementing the mechanism investigation of *B. subtilis* spore inactivation by UV–TiO_2.

The objective of this study was to contrast the potential inactivation effects of UV, TiO_2 and the simultaneous process of UV–TiO_2. *B. subtilis* spore was selected as the indicator microorganism. To receive a better understanding of mechanisms, the impact of photocatalytic disinfection on lipid peroxidation was studied. Malondialdehyde (MDA), the degradation product of lipid peroxidation, was used as an index to determine the extent of the reaction. In order to observe changes in cell ultrastructure caused by the lipid peroxidation reaction, transmission electron microscope (TEM) images, with and without disinfection, were taken to provide a deep insight into the cell morphology.

1. Materials and methods

1.1. Reagents

The photocatalyst used in this study was Degussa P-25 TiO_2 (Sigma, Mannheim, Baden-Württemberg, German). Other reagents, supplied by Sinopharm Chemical Reagent Company Limited (Shanghai, China), were analytical reagent grade. Distilled water for analytical use was from Direct-Q3 (MilliPore, Boston, Massachusetts, USA). The MDA test kit used in this research was provided by Nanjing Jiancheng Biology Corporation (Nanjing, Jiangsu, China). Reagents and materials used were sterilized by autoclaving (120°C, 20 min).

1.2. Experimental apparatus

A Collimated Beam apparatus with a 75 W low-pressure (LP) mercury lamp (Philips, Amsterdam, Dutch) was utilized (Fig. 1). The wavelength of the UV lamp used in this method was 253.7 nm. The monochromatic UV emitted by the LP lamp was directed to the surface of the test samples by the collimator. The average irradiance in the solution and the UV dose were determined based on the Bolton and Linden protocol (Bolton and Linden, 2003), using a UV-M radiometer (Beijing Normal University Experiment Company, Beijing, China). In addition, several parameters, including solution volume, solution absorbance and distance from the lamp to the water surface, were taken into account following the Excel spreadsheets available at www.iuva.org. The irradiances detected at the solution surface were 0.18, 0.10, 0.008 mW/cm^2, respectively.

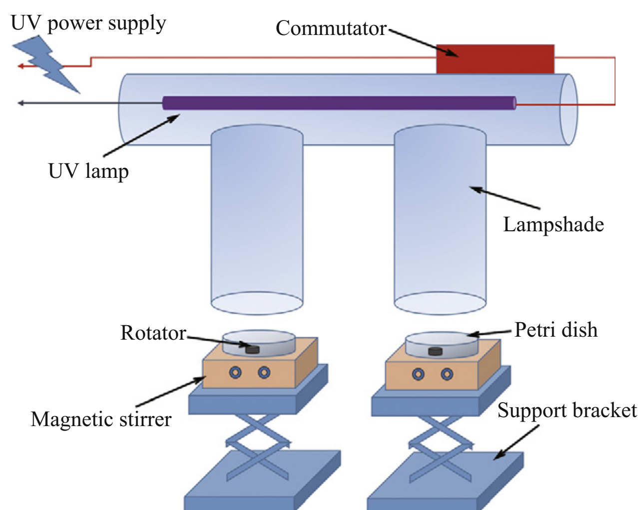

Fig. 1 – Schematic diagram of collimated beam apparatus.

1.3. *B. subtilis* spore culturing and enumeration

B. subtilis spores (ATCC 9372) (China General Microbiological Culture Collection Center, Beijing, China) were obtained freeze-dried and rehydrated aseptically with Nutrient Broth (Sinopharm Chemical Reagent Company Limited, Shanghai, China). Broth cultures were incubated in a shaker at 37°C for 24 hr and then in sporulation medium at 37°C for 48 hr. The cultures were purified by centrifugation (6000 r/min, 10 min), redissolved in physiological salt solution and heat shocked (80°C, 10 min). After disinfection, the spores were serially diluted and injected onto nutrient agar medium to enumerate, according to the method described by Liu and Zhang (2006).

1.4. Experimental methods

Petri dishes (90 mm diameter) containing 40 mL water samples were exposed to UV and stirred gently by a magnetic stir bar. TiO_2 was spiked into the samples to obtain concentrations of 5 and 10 mg/L. The UV–TiO_2 process was performed based on UV exposure with the addition of TiO_2. Determined by the thiobarbituric acid (TBA) method (Ganhao et al., 2011) with a DR 5000 spectrophotometer (HACH, Loveland, Colorado, USA), the MDA content was expressed as nmol/mg of cell dry weight (Maness et al., 1999). The TEM samples with and without treatment were prepared following the methods described by Ou et al. (2011) and examined using a JEM-1230 TEM (JEOL, Tokyo, Japan).

1.5. Data presentation

To evaluate the effect of disinfection, the inactivation level of *B. subtilis* spores is usually analyzed by the inactivation effect (Hijnen et al., 2006), described as follows:

$$-\log(N/N_0) = kt \tag{1}$$

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