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Photocatalytic inactivation of Gram-positive and Gram-negative bacteria using fluorescent light

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Abstract

A batch photocatalytic study was carried out to inactivate six different species of bacteria using fluorescent light and TiO_2 photocatalyst. Several surface loadings of TiO_2 varying from 234 to 8662 mg/m^2, impregnated on membrane filters were used with fluorescent light of constant illuminance of 3900 lux for the inactivation of four ATCC bacteria (*Escherichia coli* K-12, *Pseudomonas fluorescens*, *Bacillus subtilis* and *Microbacterium* sp.) and two other species of bacteria collected from outdoor air in Singapore (*Microbacteriaceae* str. W7 and *Paenibacillus* sp. SAFN-007). Gram-negative bacterium *E. coli* K-12 was the most effectively inactivated, while Gram-positive *B. subtilis* exhibited the least response to the photocatalytic treatment. The inactivation rate increased with an increase in the TiO_2 loading, the maximum inactivation of most bacteria was achieved at an optimum TiO_2 loading of 511–1666 mg/m^2, corresponding to a thickness of 294–438 nm of TiO_2 layer on the surface. 100% of the *E. coli* K-12 was inactivated after 30 min of treatment at a TiO_2 loading of 1666 mg/m^2, while inactivation of 1 $\log_{10}$ was obtained for *Microbacterium* sp., *Paenibacillus* sp. SAFN-007 and *Microbacteriaceae* str. W7 after 2 h of illumination at a TiO_2 loading of 1116 mg/m^2.

Keywords: TiO_2; Fluorescent light; Inactivation rate; *E. coli* K-12; *Pseudomonas fluorescens*; *Bacillus subtilis*; *Microbacterium* sp.; *Microbacteriaceae* str. W7; *Paenibacillus* sp. SAFN-007

1. Introduction

Microbial pollutants are one of the significant sources of indoor air pollution. They consist of particles of biological origin (such as bacteria, viruses, fungi) and can be airborne as bioaerosols [1]. Among the control methods, which have been employed to combat the adverse health effects of indoor biopollutants, such as purging indoor air with outside air, filtering out the microbial species, isolation by pressurization control, inactivation using low-level ozonation and ultraviolet germicidal irradiation (UVGI) [2], the effect of ultraviolet radiation on damaging bacterial cells have been well established for water disinfection [3,4].

Inactivation of bacteria by heterogeneous photocatalysis using UV-A (320–400 nm) with TiO_2 has been envisaged as one of the most effective disinfection technologies, since no carcinogenic, mutagenic or malodorous compounds are formed during the process [5]. Upon irradiation with photons of wavelength $\leq$385 nm, TiO_2 promotes an electron transfer from the valence band to the conduction band, thus forming an electron–hole pair. The photo-generated holes and electrons react with water attached to TiO_2 surface and oxygen in water respectively to form hydroxyl radicals ($^\bullet$OH) and other reactive oxygen species (ROS), such as $O_2^{\bullet-}$, $HO_2^\bullet$ and H_2O_2 [6]. The hydroxyl radicals are highly reactive with organic substance [7]. The bactericidal efficiency of heterogeneous photocatalysis has been tested on various bacterial species, such as *Escherichia coli* K-12 [5,7–9], *E. coli* [10–13], *Bacillus subtilis* [14], *Staphylococcus aureus*, *Enterococcus faecium* [13], *Enterobacter cloacae* [5], *Pseudomonas aeruginosa* [5,13], *Salmonella typhimurium* [5], etc. While heterogeneous photocatalysis using UV-A and TiO_2 has been proven to be successful in the treatment of water, the application of inactivating air-borne bacteria is relatively new. Most studies cited earlier inactivated bacteria using either a TiO_2 sus-

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pension [12,15] or TiO_2 immobilized on a support, such as glass [9,13,16] or quartz disc [14].

When microbes with high molecular weight are deposited by contact or in the form of aerosols on photocatalytic surfaces, their residence time on the surface can be considered to be infinite, unless they are removed physically or chemically [14]. Determining the time taken to oxidatively remove bacterial mass from any surface can be important for the design of sterile rooms and surfaces, which requires systematic inactivation studies. While using UV-A coupled with TiO_2 can be an effective method, the inactivation efficiency of bacteria using common fluorescent light requires dedicated parametric studies, because indoor environments (such as commercial and office premises) have TiO_2 as the key constituent of wall paints, and are commonly illuminated by fluorescent light.

A fluorescent lamp can emit a very small fraction of UV-A [17], because it is essentially a low-pressure mercury lamp with the inner surface coated with various types of phosphors to absorb the 254 nm radiation and emit longer wavelengths [18]. Although the glass envelope surrounding the lamp absorbs all far-UV emission, the commonly used daylight or cool white lamps radiate appreciable amounts at 313, 334, and 365 nm of the mercury lines. A much stronger emission at these wavelengths is typical of the blacklight lamps, which are sometimes used in rooms to provide a fluorescent effect.

For optimum utilization of the existing lighting in indoor environments, and to minimize additional energy consumption, this study systematically examines the effect of inactivation of bacteria using fluorescent light and TiO_2 photocatalysts, and is a part of an on-going study, where fluorescent light is being used to inactivate bioaerosol in a continuous mode. To our best knowledge, the inactivation of bacteria using TiO_2 catalyst irradiated by fluorescent light has not been reported in the literature. In this study, the effect of TiO_2 loading on the inactivation efficiency of six different bacterial strains under fluorescent irradiation has been evaluated. Selected bacteria, *E. coli* K-12, *B. subtilis* and *Microbacterium* sp., were also tested using UV-A radiation for a comparison.

2. Materials and methods

2.1. Materials

The non-porous titanium dioxide (TiO_2, P25, Degussa AG, Germany) used as the photocatalyst had a primary particle diameter of 21 nm, specific surface area of $50 \pm 15\ m^2/g$, and a crystal distribution of 80% anatase and 20% rutile. TiO_2 suspensions in deionised water at nine different concentrations were prepared and autoclaved for the following inactivation experiments.

E. coli K-12 (ATCC 10798), *Pseudomonas fluorescens* (ATCC 17575), *Microbacterium* sp. (ATCC 15283), *B. subtilis* (ATCC 14410), *Microbacteriaceae* str. W7 and *Paenibacillus* sp. SAFN-007 were used for the inactivation studies. The former four species were purchased from ATCC, while the latter two species were collected from outdoor air in Singapore using a six stage sampler (Andersen, Atlanta, GA, USA) and identified to their respective closest relatives. *E. coli* and *P. fluorescens* are Gram-negative bacteria, while the rest are Gram-positive.

In the photocatalytic experiments, an 18 W fluorescent lamp (NEC 6700K, TRI-PHOSPHOR T8, Japan), which is commonly used for room illumination with a wavelength range of 400–700 nm, was used as the light source. The fluorescent illuminance (3900 lux) was monitored using a luxmeter through the experiments. In addition to fluorescent lamps, two blacklight blue lamps (FL8BLB, Sanyo Denki, Japan) with a peak emission at 365 nm and an energy output of 8 W each were used as the light source for UV-A photocatalysis. A digital radiometer was used to determine the intensity of the UV-A light source of the blacklight blue lamps and the fluorescent lamp.

2.2. Bacterial culture preparation

Bacterial cells were inoculated in 10 ml of a Luria-bertani broth and incubated for 16 h at 121 rpm in a rotating water shaker at 26 and 37 °C for *P. fluorescens* and the other strains, respectively. The cultured bacteria were centrifuged at 4000 rpm for 5 min before washing with an autoclaved 0.9% sodium chloride solution twice and re-suspended in 50 ml of an autoclaved 0.9% sodium chloride solution. The initial bacterial solution was then successively diluted to 10^7 or 10^8 times using a 0.9% sodium chloride solution in order to achieve an average of 50 colonies on the Petri dish for a 50 ml solution used for incubation. Between each dilution, the bacterial suspension was well stirred using a vortex mixer to ensure uniformity of the suspension.

2.3. Membrane filter preparation

For the control experiments without TiO_2, a 50 ml aliquot of the earlier prepared bacterial solution was filtered through a cellulose acetate membrane filter (with an average pore size of 0.45 μm and a diameter of 47 mm) before the filter was placed in a sterile Petri dish. For the inactivation experiments with TiO_2, 50 ml of the autoclaved TiO_2 solution at a required concentration was first filtered, followed by immobilizing the bacterial suspension onto the TiO_2-loaded filters. To determine the amount of TiO_2 coated on each membrane filter, the membrane filters were weighed before and after the TiO_2 impregnation process followed by drying and desiccation. Five tests showed an average TiO_2 loading ranging from 234 to 8662 (mg/m^2), depending on the initial TiO_2 suspension employed (Table 1). The uniformity of the TiO_2 coating over the membrane filter surface was examined using scanning electron microscopy (SEM); Fig. 1 shows that although some agglomeration was observed at a high TiO_2 loading of 5778 mg/m^2 (Fig. 1d), Fig. 1b and c show a relatively homogeneous TiO_2 coating. The pore size of the coated membrane filters is expected to be reduced to less than 0.45 μm, which can completely eliminate any chance of bacteria (≈1 μm) escaping through the pores of the filters. Assuming a uniform distribution of TiO_2, the respective thickness of the TiO_2 coating on each membrane was calculated by dividing the TiO_2 loading on each filter by the specific gravity of TiO_2. The coating thickness ranged from 62 to 2279 nm for individual TiO_2 loadings (last column, Table 1).