Available online at www.sciencedirect.com
ScienceDirect
Journal of Photochemistry and Photobiology A: Chemistry 186 (2007) 335–341
Journal of Photochemistry and Photobiology A:Chemistry
www.elsevier.com/locate/jphotochem

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

Amrita Pal [a], Simo O. Pehkonen [b], Liya E. Yu [b], Madhumita B. Ray [c,*]

[a] *Department of Chemical and Biomolecular Engineering, National University of Singapore, Singapore*
[b] *Division of Environmental Science & Engineering, National University of Singapore, 4 Engineering Drive 4, Singapore 117576, Singapore*
[c] *Department of Chemical and Biochemical Engineering, Thompson Engineering Building, Room 443, Faculty of Engineering, The University of Western Ontario, London, Ontario N6A 5B9, Canada*

Received 7 February 2006; received in revised form 29 August 2006; accepted 1 September 2006
Available online 2 October 2006

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-

* Corresponding author. Tel.: +1 519 661 2111x81273; fax: +1 519 661 3498.
E-mail address: mray@eng.uwo.ca (M.B. Ray).

1010-6030/$ – see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.jphotochem.2006.09.002

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).

Table 1
TiO_2 loading and the resulting thickness of the TiO_2 coating on the membrane

TiO_2 concentration		Error in the loading (%)	Thickness of the TiO_2 coating on the membrane (nm)[c]
In suspension (mg/l[a])	Loading on the membrane filter surface (mg/m^2)[b]		
10	234	5.12	62
20	511	6.56	134
30	840	14.82	221
40	1116	5.35	294
60	1666	6.49	438
80	2297	2.03	605
120	3490	1.13	919
200	5778	1.04	1521
300	8662	0.61	2279

The error is based on a replicate of five sets of data.
[a] The amount of the TiO_2 solution impregnated on each membrane is 50 ml.
[b] The surface area of the membrane, on which TiO_2 is being loaded, is 17.35 cm^2.
[c] The specific gravity of TiO_2 has been taken as 3.8.

2.4. Bacteria inactivation using fluorescent irradiation

Fig. 2 shows the schematic set-up of the batch inactivation system. The set-up comprised of an irradiation source clamped on top of a flexible support holding the bacterial samples in Petri dishes (three replicates) and placed inside a black-box to minimize the penetration of light from outside. While the air inside the black-box is same as the air in the laboratory, the air is not in direct contact with the bacterial samples. With the lamp position at 8–9 cm above the bacteria impregnated on the filter membrane, the samples were exposed to an UV-A (365 nm) intensity of 4.28 and 0.013 mW/cm^2 under blacklight blue lamps and fluorescent lamp, respectively.

Six irradiation durations of 0, 15, 30, 45, 60 and 120 min were employed to examine the efficiency of the photocatalytic inactivation. Triplicate measurements were taken for each experiment. The temperature and the relative humidity in the black-box, which were measured before and after individual experiments, were reasonably constant throughout the experiment. After irradiation, filters were immediately removed from the Petri dishes and placed face-down on agar plates. For *E. coli* K-12 and *P. fluorescens*, eosin methylene blue (EMB) agar plates were used, while tryptic soy agar (TSA) was used for *Microbacterium* sp.,

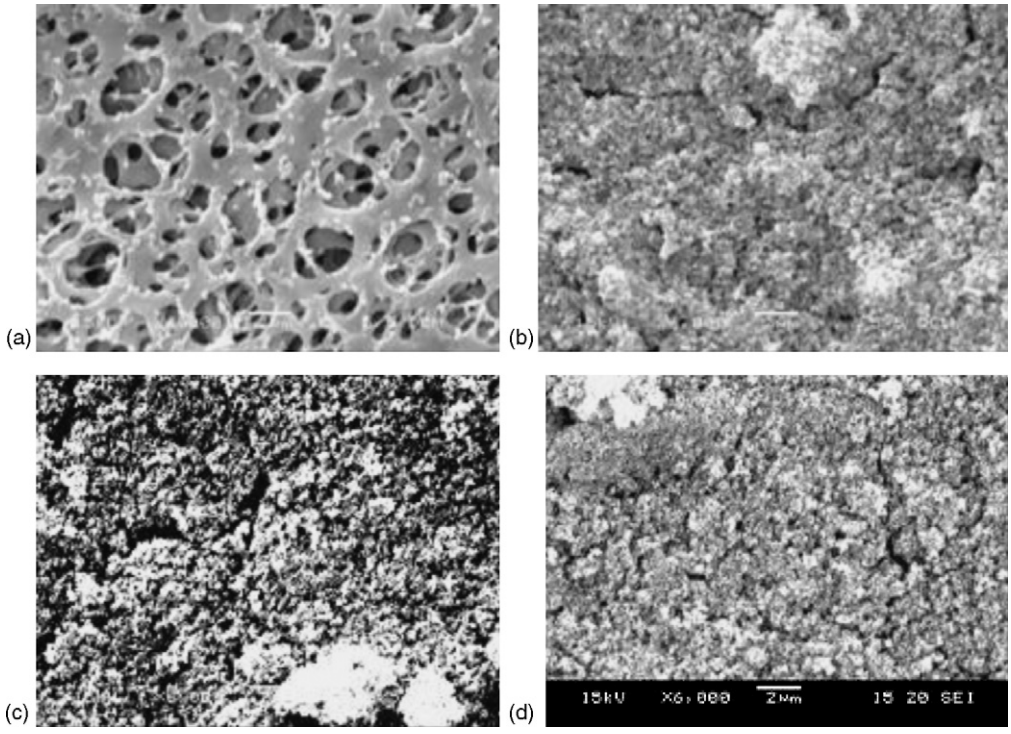

Fig. 1. SEM images (with a 6000× magnification and 2 μm resolution) of the membrane filters with a TiO_2 loading of (a) 0 mg/m^2, (b) 1116 mg/m^2, (c) 3490 mg/m^2, and (d) 5778 mg/m^2.

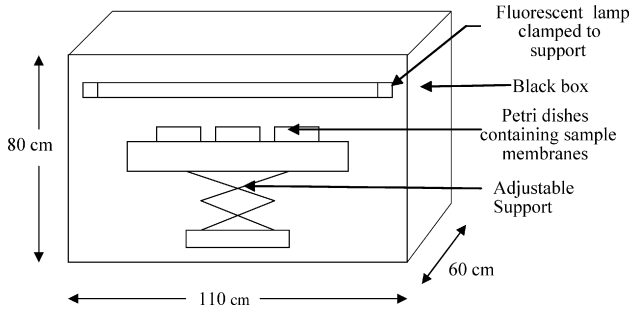

Fig. 2. Schematic diagram of the batch experimental set-up.

Paenibacillus sp. SAFN-007 and *B. subtilis*, and R2A agar was used for *Microbacteriaceae* str. W7. All the plates were sealed with wax tapes and placed in an incubator at 26 and 37 °C for *P. fluorescens* and the other bacterial strains, respectively. To monitor the regrowth of the survived colonies, the colonies were then counted daily using a colony counter on days 2 through 5, and on the 10th day. For all the species, the growth of colonies was complete within 3 days of incubation, but in just one among an average of six experiments, *E. coli* and *Microbacterium* sp. showed new growth of colonies in 2–3 of the 18 Petri dishes on the fifth day. However, after 5 days, no new bacterial colony was observed; it is evident that the revival of bacteria exposed to heterogeneous photocatalytic inactivation after 5 days in dark was insignificant, which can be due to irreversible cell damage. To determine the background interference, two types of control experiments were carried out; one was conducted without the light source, and the second type of control experiment was conducted with light, but in the absence of TiO_2.

The bacterial inactivation efficiency followed first order kinetics with respect to bacterial colony count (N_t), which is shown by Eq. (1):

$$\ln\left(\frac{N_t}{N_0}\right) = -kt \tag{1}$$

where, N_t is the number of CFUs after irradiation for t min; N_0 the number of CFUs at 0 min; k the inactivation rate constant; N_t/N_0 the survival ratio.

The survival ratio was calculated by normalizing the resultant CFUs on any plate to that on the plate without exposure to light. This ratio was compared under different durations of exposure to photocatalytic treatment and catalyst loadings to determine the inactivation efficiencies.

3. Results and discussion

3.1. Bacteria inactivation under fluorescent irradiation without TiO_2

Six different control experiments exposing the six immobilized bacterial strains with 1116 mg/m^2 of TiO_2 to a dark environment up to 2 h showed that the average colony counts for all the six bacterial strains varied insignificantly with a standard deviation of 0–10% (not shown). Hence, in the absence of fluorescent light, bacteria impregnated on a membrane surface

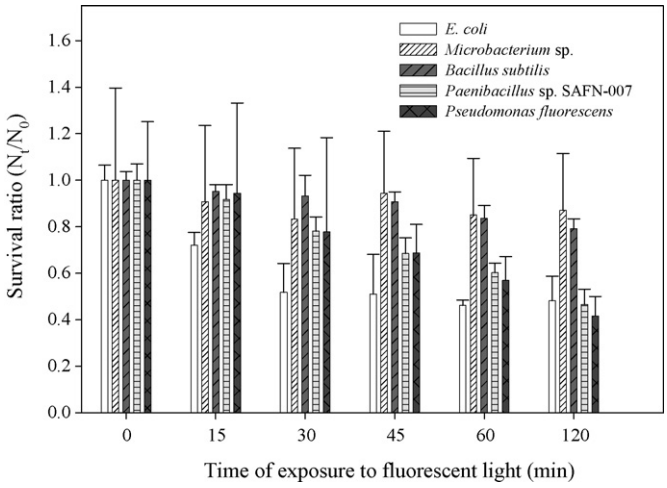

Fig. 3. Survival ratios (N_t/N_0) as a function of exposure duration (without TiO_2) for *E. coli* K-12, *P. fluorescens*, *Microbacterium* sp., *Paenibacillus* sp. SAFN-007 and *B. subtilis*. The data shown in this figure are the averages of three replicates. Here, N_t is the number of CFUs after irradiation for t min; N_0 the number of CFUs at 0 min.

seemed to be insensitive to TiO_2. Without TiO_2, the exposure to fluorescent irradiation appeared to inactivate various strains of bacteria. Fig. 3 shows that in 2 h, around 40–50% of *E. coli* K-12, *P. fluorescens* and *Paenibacillus* sp. SAFN-007, were inactivated. Although *Microbacteriaceae* str. W7 showed negligible inactivation (data not shown), 20% of *B. subtilis* and only 13% of *Microbacterium* sp. were inactivated. However, considering the error bars for *Microbacterium* sp. shown in Fig. 3, inactivation of this species is practically nil in the absence of TiO_2.

The observed inactivation in the absence of TiO_2 could be due to the small fraction of UV-A emitted from the fluorescent light; it has been reported that exposure to UV-A can form reactive oxygen radicals within the cells, which cause oxidative stress and lead to cell damage [13]. In addition, long wavelength UV light (i.e., 320–400 nm) has been reported to damage organisms mainly by exciting photosensitive molecules within the cell, thus producing active species, such as $O_2^{\bullet-}$, H_2O_2, and $^{\bullet}OH$, to adversely affect the genome and other intracellular molecules sublethally or lethally causing cell mutations, growth delay, etc. [19].

3.2. Bacteria inactivation under fluorescent irradiation and TiO_2

As mentioned earlier, the inactivation of bacteria in the presence of TiO_2 irradiated by fluorescent light exhibited first order reaction kinetics. The extent of inactivation increased with increased exposure to fluorescent light for all the bacteria. However, individual bacteria show different trend in the inactivation rate constant with increasing TiO_2 loading.

Because *E. coli* is the most investigated species with known chromosome map in detail [17], to compare and validate our experimental approaches with the published literature, *E. coli* K-12 was selected to examine the inactivation efficiency of fluorescent light irradiated TiO_2 catalysts. Fig. 4 shows the inactivation of *E. coli* K-12 at 0, 15, 30, 45, 60 and 120 min with six

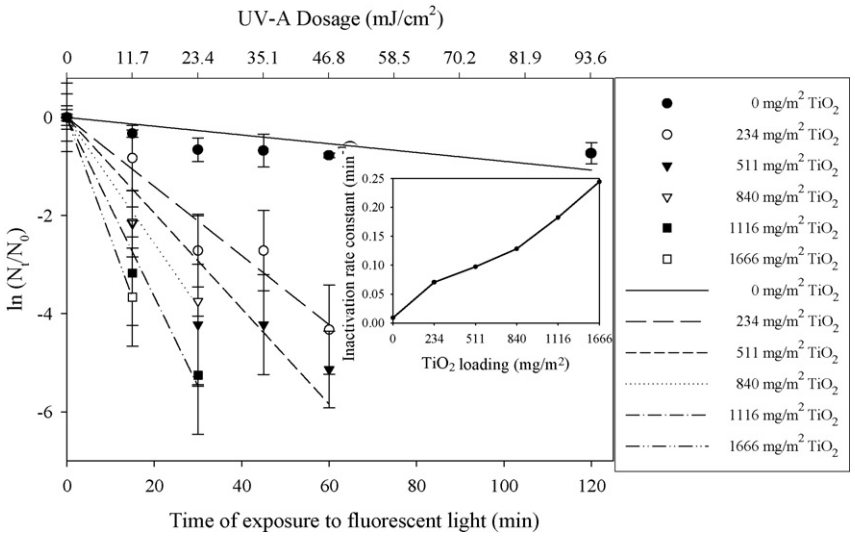

Fig. 4. Photocatalytic inactivation of *E. coli* K-12 at various TiO_2 loadings. Each point is an average of triplicate experiments.

UV-A dosages (0, 11.7, 23.4, 35.1, 46.8 and 93.6 mJ/cm^2). An increase in the TiO_2 loading from 234 to 1666 mg/m^2 enhanced the inactivation efficiency of *E. coli* K-12 (Fig. 4). With a TiO_2 loading of 234 mg/m^2, over 98% of *E. coli* K-12 was inactivated within 60 min (46.8 mJ/cm^2) of fluorescent irradiation. When a larger TiO_2 loading of 1666 mg/m^2 was employed, over 96% of the bacteria were inactivated within 15 min of exposure, and all the bacteria were inactivated after a 30 min or longer exposure. This is encouraging because a UV-A intensity of only 0.013 mW/cm^2 available in the fluorescent irradiation yielded an inactivation efficiency comparable to that achieved by Huang et al. [11], who reported the damage of cell walls of *E. coli* within 20 min of exposure to UV-A light at 0.8 mW/cm^2 in presence of TiO_2.

Because a higher TiO_2 loading may enhance the generation of reactive oxygen species (ROS) to react with cell walls, the cytoplasmic membrane, and other intracellular components, the resultant inactivation rate was substantially increased. This is consistent with previous studies reporting that higher inactivation of *E. coli* in an aqueous medium was observed when higher TiO_2 concentrations were used with UV–vis radiation longer than 380 nm [20], or under UV-A irradiation [21]. It is interesting to note that in this study, photon limitation did not occur at a high TiO_2 loading of 1666 mg/m^2 with corresponding thickness of 238 nm of TiO_2 on the surface.

3.3. Inactivation of all bacteria

Fig. 5 shows that most Gram-negative bacteria were inactivated with a TiO_2 loading of less than 2000 mg/m^2, while the Gram-positive bacteria appeared to be more resistant to the fluorescent light-TiO_2 induced damage (Fig. 5a). *E. coli* K-12 and *P. fluorescens* (i.e., Gram-negative bacteria) showed the highest inactivation rate (0.0078–0.2442 min^{-1}) among all the tested bacterial strains, which agrees well with other studies that the inactivation efficiency of Gram-negative bacteria, such as *E. coli*, was higher than that of Gram-positive bacteria *S. aureus*,

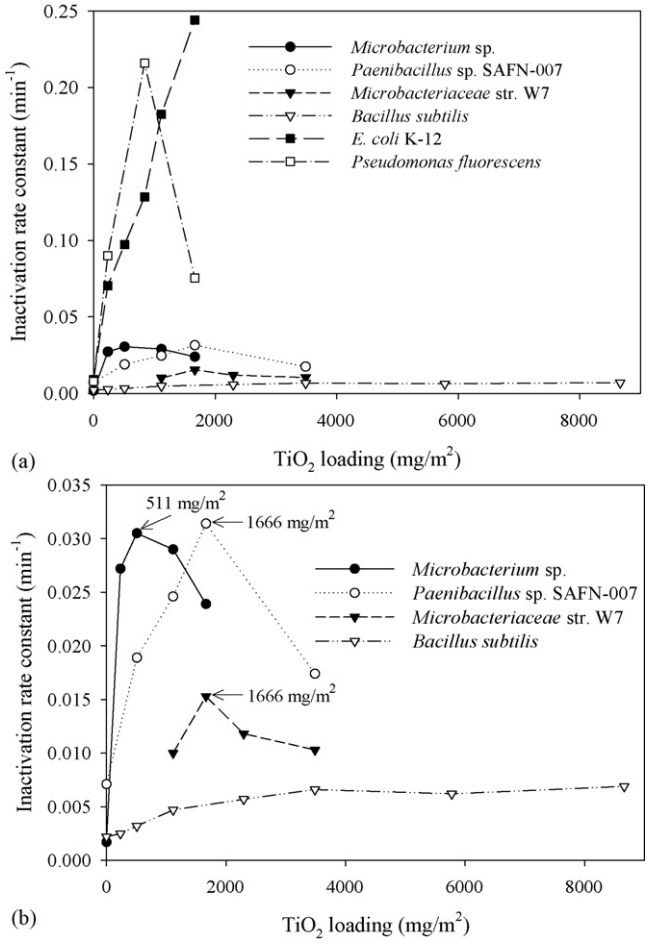

Fig. 5. (a) Inactivation rate constant vs. the TiO_2 loading for Gram-negative and Gram-positive bacteria and (b) inactivation rate constant vs. the TiO_2 loading for Gram-positive bacteria, enlarged from (a).

E. faecium [13], and *L. helveticus* [12]. Gram-positive bacteria have a thick mesh-like cell wall made of peptidoglycan, while Gram-negative bacteria have a thin cell wall made of a layer of peptidoglycan. In addition to an inner membrane, they also have an outer membrane, which contains lipids, and is separated from the cell wall by the periplasmic space. Although cell wall structure of Gram-negative bacteria is relatively more complex than that of Gram-positive cells, Gram-negative bacteria responded better for photocatalytic inactivation indicating cell wall destruction is probably not necessary for inactivation. Cell membrane damage is measured as loss of potassium ions, proteins and RNA from bacterial cells or as an increase in intracellular calcium ions [12]. On the other hand, free radicals can react with the nucleic acids of the cell. It is also possible that reactive radicals are absorbed by the peptidoglycan layer of the Gram-positive bacteria without doing any fatal damage, while the opposite might be true for the Gram-negative bacteria. Interestingly, some studies have shown Gram-positive bacteria to be more easily killed than Gram-negative bacteria. For example, in a recent study involving cell killing using photosensitizers and visible light, the overall susceptibility of Gram-positive *S. aureus* was found to be greater than Gram-negative *E. coli* [22]. In another study using porphyrin-mediated photodynamic therapy, the outer membrane of Gram-negative cells rendered them completely resistant [23], while in a study based on exposure of Gram-positive and Gram-negative cells to singlet oxygen for various durations, survival of Gram-positive cells showed fast decrease compared to that of the Gram-negative cells [24]. The experimental results in this work, which agree with those of Liu and Yang [12], indicate that Gram-positive and Gram-negative cells may undergo different mechanisms of photocatalytical inactivation, warranting further research of the radical-cell chemical reactions.

Fig. 5a shows that the inactivation rate constant for *P. fluorescens* increases with an increase in the TiO_2 loading to reach an optimum at a loading of 840 mg/m^2, after which it dramatically decreases at a loading of 1666 mg/m^2. To better examine the inactivation rates among the four Gram-positive bacteria, Fig. 5b (enlarging the trends of Gram-positive bacteria shown in Fig. 5a) shows that the inactivation rate constant of Gram-positive bacteria *Paenibacillus* sp. SAFN-007 and *Microbacteriaceae* str. W7 reached a maximum at 1666 mg/m^2 with a corresponding thickness of the TiO_2 coating on the membrane of 438 nm (Table 1). Since the wavelength of UV-A light is in the range of 320–400 nm with peak wavelength of 365 nm, a thin TiO_2 coating of 62 and 134 nm may not completely absorb light of 365 nm [25], whereas a thicker TiO_2 coating in the range of 134–438 nm (Table 1), could completely absorb the incoming UV-A. Nevertheless, further increases in the TiO_2 loading with agglomeration of TiO_2 on the filter surface could reduce activation efficiency. With increasing TiO_2 concentration, terminal reactions such as reactions (3) and (4) shown below would cause the formation of less reactive hydroperoxyl radicals ($HO_2^\bullet$) and decrease the bacterial inactivation efficiency [7].

$$^\bullet OH + {}^\bullet OH \rightarrow H_2O_2 \quad (3)$$

$$H_2O_2 + {}^\bullet OH \rightarrow H_2O + HO_2^\bullet \quad (4)$$

Interestingly, unlike other Gram-positive bacteria shown in Fig. 5b, the inactivation rate constant of *B. subtilis* (in hollow triangle symbols) reached a plateau at a loading higher than 2297 mg/m^2 (Fig. 5b). Although in the absence of TiO_2, 2 h of exposure to fluorescent light inactivated ~21% of the *B. subtilis* (Fig. 3), the presence of TiO_2 with loadings up to 8662 mg/m^2 only inactivated around 53% of the bacteria (data not shown) suggesting that *B. subtilis* were little affected by either fluorescent (with a small amount of UV-A) irradiation or increased TiO_2 loading. This can be supported by Kuhn et al. [13], who reported that the spores of *B. subtilis* were well resistant to 60 min of UV-A photocatalytic treatment. It is interesting to note that *Microbacteriaceae* str. W7 did not show inactivation until a high TiO_2 loading of 1116 mg/m^2 was employed, indicating that this bacterium is highly resistant to photocatalytic inactivation (Fig. 5b). For *Microbacterium* sp. and *Microbacteriaceae* str. W7, 1 $\log_{10}$ inactivation was obtained after 2 h of light exposure at all TiO_2 loadings; the same level inactivation of *Paenibacillus* sp. SAFN-007 occurred in the TiO_2 loading range of 1116–1666 mg/m^2, while 100% inactivation of *P. fluorescens* was obtained after 45 min of exposure to light at a TiO_2 loading of 840–1666 mg/m^2 (data not shown).

3.4. Comparison of fluorescent light inactivation with UV-A inactivation

Limited experiments were carried out using UV-A at intensity of 4.28 mW/cm^2 for Gram-negative *E. coli* K-12 and Gram-positive *B. subtilis* and *Microbacterium* sp., all purchased from ATCC. Table 2 shows that regardless of the light sources, the inactivation rate constant increases with an increase in the TiO_2

Table 2
Inactivation rate constants of *E. coli*, *Microbacterium* sp. and *B. subtilis* using UV-A and fluorescent light photocatalysis

TiO_2 loading (mg/m^2)	First-order inactivation rate constants (k) in min^{-1}					
	Fluorescent light inactivation (0.013 mW/cm^2 of UV-A)			UV-A inactivation (4.28 mW/cm^2)		
	E. coli K-12	*B. subtilis*	*Microbacterium* sp.	*E. coli* K-12	*B. subtilis*	*Microbacterium* sp.
0	0.009	0.0022	0.005	0.175	0.1279	0.171
234	0.070	0.0027	0.027	0.180	0.1263	0.238
511	0.097	0.0032	0.031	0.243	0.1807	0.262
840	0.129	–[a]	0.018	0.290	0.1979	–[b]

[a] Data not available.
[b] Experimental error.

loading (up to 840 mg/m^2), although the fluorescent light (with a small UV-A intensity of 0.013 mW/cm^2) yielded inactivation rate constants much smaller than UV-A inactivation (at a light intensity of 4.28 mW/cm^2). This is expected because the UV-A intensity was about 330 times higher than the UV-A available in the fluorescent light. Although both *B. subtilis* and *Microbacterium* sp. were more resistant to photocatalytic treatment than *E. coli* K-12, they were more greatly affected by the higher UV-A intensity (Table 2), and the effect was slightly higher in the absence of TiO_2 (loading of 0 mg/m^2). In the presence of TiO_2, the inactivation rates were higher by a factor of 2.57–56, in particular for the Gram-positive bacteria (*B. subtilis* and *Microbacterium* sp.) (Table 2). These results indicate two possibilities: (i) the reactions of $OH^{\bullet}$ radical and other reactive species may slightly inactivate the Gram-positive bacteria compared to direct damage by exciting photosensitive molecules within cells, (ii) 0.013 mW/m^2 of UV-A light available in the fluorescent light is sufficient to excite the TiO_2 loading used in this study.

By employing a UV-A intensity of 5.5 mW/cm^2 and a TiO_2 suspension of 0.1 g/l, Ibanez et al. [5] obtained a first-order inactivation rate constant of 0.29 min^{-1} for *E. coli* K-12. These rate compares well with the rate obtained in this work (Table 2) using TiO_2 concentration of 0.03 g/l (which corresponds to a 840 mg/m^2 loading on the membrane surface) and UV-A intensity of 4.28 mW/cm^2, indicating the consistency of the UV-A photocatalytic inactivation of the microorganisms, and the possibility of scaling-up the process. Photocatalytic inactivation experiments on *E. coli* and *Microbacterium* sp. at a UV-A intensity of 0.013 mW/cm^2 are currently in progress to understand whether only the UV-A fraction in the fluorescent light is responsible for bacterial inactivation.

4. Conclusions

TiO_2 mediated inactivation of bacteria is possible in the presence of fluorescent light, commonly used as room lighting. Experiments on six strains of bacteria including four Gram-positive and two Gram-negative bacteria have shown that photocatalytic inactivation was most effective on *E. coli*, while it was least effective on *B. subtilis*. Four bacteria showed the maximum inactivation at an optimum TiO_2 loading ranging from 511 to 1666 mg/m^2. Complete inactivation of *E. coli* was achieved after 30 min of fluorescent irradiation at a TiO_2 loading of 1666 mg/m^2. This loading of TiO_2 can be applied to indoor walls commonly illuminated with fluorescent lighting to induce inactivation of the indoor microbes. In this study, the inactivation rates using TiO_2 and UV-A of intensity 0.013 mW/cm^2, ranged from 0.0017 to 0.2442 min^{-1}, consistent with limited data available in the literature, indicating the potential for large-scale application of the process. In contrast to the Gram-negative bacteria, reaction with OH radicals and ROS could cause limited inactivation of Gram-positive bacteria. The revival of inactivated bacteria after 5 days of incubation was insignificant, which is due to the irreversible cell damage of the bacteria due to the reactions with OH radicals and other reactive oxygen species. Based on the kinetic data from this study, a systematic study involving inactivation of aerosolized bacteria using fluorescent light in a continuous reaction set-up is currently in progress.

Acknowledgement

The authors acknowledge the financial support from National University of Singapore, grant RP-279-000-131-112.

References

[1] J. Lacey, J. Dutkiewicz, J. Aerosol Sci. 25 (1994) 1371–1404.
[2] W.J. Kowalski, W. Bahnfleth, HPAC Eng. 70 (1998) 34–47.
[3] F. Taghipour, Water Res. 38 (2004) 3940–3948.
[4] W.A.M. Hijnen, E.F. Beerendonk, G.J. Medema, Water Res. 40 (2006) 3–22.
[5] J.A. Ibanez, M.I. Litter, R.A. Pizarro, J. Photochem. Photobiol. A: Chem. 6283 (2003) 1–5.
[6] N. Huang, Z. Xiao, D. Huang, C. Yuan, Supramol. Sci. 5 (1998) 559–564.
[7] A.G. Rincón, C. Pulgarin, Appl. Catal. B: Environ. 44 (2003) 263–284.
[8] P.C. Maness, S. Smolinski, D.M. Blake, Z. Huang, E.J. Wolfrum, W.A. Jacoby, Appl. Environ. Microbiol. 65 (1999) 4094–4098.
[9] W.A. Jacoby, P.C. Maness, E.J. Wolfrum, D.M. Blake, J.A. Fennell, Environ. Sci. Technol. 32 (1998) 2650–2653.
[10] M. Bekbolet, C.V. Araz, Chemosphere 32 (1996) 959–965.
[11] Z. Huang, P. Maness, D.M. Blake, E.J. Wolfrum, S.L. Smolinski, W.A. Jacoby, J. Photochem. Photobiol. A: Chem. 130 (2000) 163–170.
[12] H. Liu, T.C. Yang, Process Biochem. 39 (2003) 475–481.
[13] K.P. Kuhn, I.F. Chaberny, K. Massholder, M. Stickler, V.W. Benz, H. Sontag, L. Erdinger, Chemosphere 53 (2003) 71–77.
[14] E.J. Wolfrum, J. Huang, D.M. Blake, P. Maness, Z. Huang, J. Fiest, Environ. Sci. Technol. 36 (2002) 3412–3419.
[15] T. Matsunaga, R. Tomoda, T. Nakajima, H. Wake, FEMS Microbiol. Lett. 29 (1985) 211–214.
[16] K. Sunada, T. Watanabe, K. Hashimoto, J. Photochem. Photobiol. A: Chem. 156 (2003) 227–233.
[17] W. Harm, Biological Effects of Ultraviolet Radiation, Cambridge University Press, 1980.
[18] J. Bolton, Fundamentals of ultraviolet light, First Asia Regional Conference on Ultraviolet Technologies for Water, Wastewater & Environmental Applications, 2002.
[19] K. Oguma, H. Katayama, S. Ohgaki, Appl. Environ. Microbiol. 68 (2002) 6029–6035.
[20] C. Wei, W. Lin, Z. Zainal, N. Williams, K. Zhu, A.P. Kruzic, Environ. Sci. Technol. 28 (1994) 934–938.
[21] M. Cho, H. Chung, C. Wonyong, J. Yoon, Water Res. 38 (2004) 1069–1077.
[22] T.N. Demidova, M.R. Hamblin, Antimicrob. Agents Chemother. 49 (2005) 2329–2335.
[23] Z. Malik, H. Ladan, Y. Nitzan, J. Photochem. Photobiol. B: Biol. 14 (1992) 262–266.
[24] G. Valduga, G. Bertoloni, E. Reddi, G. Jori, J. Photochem. Photobiol. B: Biol. 21 (1993) 81–86.
[25] D. Chen, F. Li, A.K. Ray, Catal. Today 66 (2001) 475–485.