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Tio_2 Mediated Photocatalytic Inactivation of Gram-Positive And Gram-Negative Bacteria Using Fluorescent Light

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Abstract

Photocatalytic inactivation of six different species of bacteria using fluorescent light and TiO_2 was conducted. Up to five surface loadings of TiO_2 varying from 234-8662 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 (*E. coli* K-12, *Pseudomonas fluorescens*, *Bacillus subtilis* and *Microbacterium* sp.) and two other species of bacteria (*Microbacteriaceae* str. W7 and *Paenibacillus* sp. SAFN-007) collected from outdoor air in Singapore. A Gram-negative bacterium *E. coli* K-12 was the most effectively inactivated, while Gram-positive *Bacillus 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 1116-1666 mg/m^2. 100% of the *E. coli* K-12 was inactivated after 30 minutes of treatment at a TiO_2 loading of 1666 mg/m^2, while inactivation of one $\log_{10}$ was obtained for *Microbacterium* sp., *Paenibacillus* sp. SAFN-007 and *Microbacteriaceae* str. W7 after two hours. Preliminary experiments indicate that the photocatalytic inactivation using Degussa P25 is 1.83-5.41 times higher than that of Hombikat UV-100.

Keywords: Photocatalytic inactivation; Gram-positive and Gram-negative Bacteria; Fluorescent light inactivation; Optimum TiO_2 loading

1.0 Introduction

Indoor air pollution due to biological contaminants (bacteria, viruses, fungi, etc.) is receiving increasing attention as a public health problem as people spend 80-90% of their time indoors. In tropical countries like Singapore, hot and humid climate enhances the proliferation and the growth of the biological contaminants in indoor environment, many of which may cause asthma and other respiratory illnesses and may transmit diseases like tuberculosis, cough and cold, mumps, measles, rubella, pneumonia, meningitis, Legionaries, influenza etc. [1].

Several control methods have been employed to combat the adverse effects of indoor bio-pollutants, such as purging indoor air with outside air, filtering out the microbiological species,

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isolation by pressurization control, inactivation using low-level ozonation and ultraviolet germicidal irradiation (UVGI) [2]. The effect of ultraviolet radiation on damaging bacterial cells and its application in water disinfection have been established in [3, 4]. Heterogeneous photocatalysis, that uses UV-A of 320-400 nm coupled with TiO_2 catalyst, is a potential alternative as the process does not involve any expensive oxidizing chemicals, uses atmospheric oxygen, and produces hydroxyl radical and reactive oxygen species which are indiscriminate and powerful oxidizing agents and have the potential of causing inactivation in most of the microorganisms [5]. Heterogeneous photocatalysis has been proved to be successful in the treatment of water to a great extent [6-11]. Application of photocatalysis to inactivate air-borne bacteria is relatively new and most of the earlier studies reported the inactivation of *E. coli* using either a TiO_2 suspension [12, 13] or TiO_2 immobilized on supports, such as glass [1, 11, 14] or quartz disc [8]. Moreover, the bactericidal efficiency of heterogeneous photocatalysis using has been tested on various bacterial species like *E. coli* K-12 [9, 7, 10, 1], *E. coli* [6, 5, 12, 11], *Bacillus subtilis* [8], *Staphylococcus aureus*, *Enterococcus faecium*, *Candida albicans* [11], *Enterobacter cloacae* [9], *Pseudomonas aeruginosa* [9, 11], and *Salmonella typhimurium* [9].

Though photocatalytic inactivation using UV-A and TiO_2 can be an effective method, the inactivation efficiency of bacteria using fluorescent light and TiO_2 requires extensive studies, since 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 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 [15] and hence can emit a very small fraction of UV-A [16]. 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. To optimize the utilization of 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. To the best of our knowledge, the inactivation of bacteria using TiO_2 catalyst irradiated by fluorescent light has not been reported in the literature. The effect of TiO_2 loading on the inactivation efficiency of six different bacterial strains under fluorescent irradiation has been evaluated.

2.0 Materials and Methods

2.1 Materials

The photocatalyst used was non-porous titanium dioxide (TiO_2, P25, Degussa AG, Germany). It had a primary particle diameter of 21 nm, specific surface area of 50 ± 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. The following bacterial strains were used for the inactivation studies: *Escherichia coli* K-12 (ATCC 10798), *Pseudomonas fluorescens* (ATCC 17575), *Microbacterium* sp. (ATCC 15283), *Bacillus subtilis* (ATCC 14410), *Microbacteriaceae* str. W7 and *Paenibacillus* sp. SAFN-007. 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, location, USA) and identified to their respective closest relatives. *Escherichia coli* K-12 and *Pseudomonas 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), was used as the light source and was clamped at 8-9 cm above the surface of the filter samples. Such lamp, with a wavelength range of 400-700 nm, is commonly used for room illumination. The fluorescent illuminance (3900 Lux) was monitored using a Luxmeter in the experiments. A digital radiometer was used to determine the intensity of the UV-A light emitted from the fluorescent lamp which was measured to be 0.013 mW/cm^2 at 365 nm on the surface of the filer. Figure 1 shows the schematic setup of the batch inactivation system.

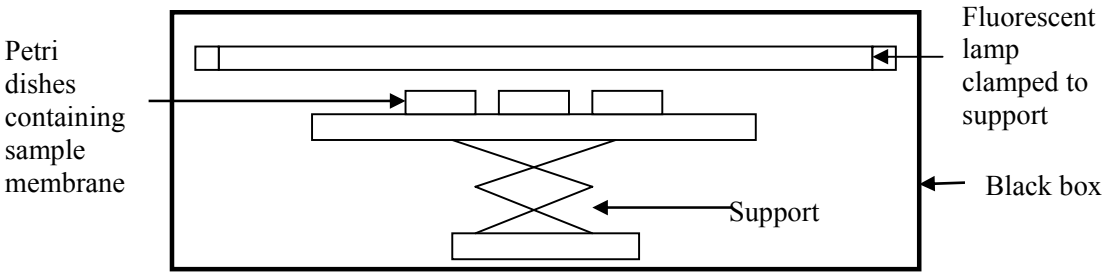

Figure 1: Schematic diagram of the batch experimental set-up

2.2 Bacterial culture and membrane filter preparation

The bacterial cells were inoculated in 10 ml of Luria-bertani broth and incubated for 16 hours at 121 rpm in a rotating water shaker at 26^{0}C and 37^{0}C for *Pseudomonas fluorescens* and the other strains, respectively. The cultured bacteria were centrifuged at 4,000 rpm for 5 minutes and washed with an autoclaved 0.9% sodium-chloride solution twice and re-suspended in 50 ml of an autoclaved 0.9% sodium chloride solution. The bacterial solution was then diluted to 10^7 or 10^8 times through consecutively re-suspending in a 0.9% sodium chloride solution and separated into individual 50 ml aliquots. Between each dilution, the bacterial suspension was well stirred using a vortex mixer to ensure uniformity of the suspension. A 50-ml aliquot of this 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) and the filter was placed in a sterile Petri dish, for the control experiments without TiO_2. In the case of the inactivation experiments with TiO_2, 50 ml of the autoclaved TiO_2 solution at a required concentration was first filtered, followed by immobilization of the bacterial suspension onto the TiO_2-loaded filters. In order 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. Five tests showed an average TiO_2 loading ranging from 234 - 8662 (mg/m^2), depending on the initial TiO_2 suspension employed (Table 1). The pore size of the coated membrane filters is expected to be reduced to less than 0.45 μm, which can ensure complete capture of the bacteria (≥1 μm) on the filters. Assuming a uniform distribution, the

thickness of the TiO_2 coating on each membrane ranged from 62-2279 nm for individual TiO_2 loadings (last column, Table 1).

Table1. TiO_2 loading and the resulting thickness of the TiO_2 coating on the membrane. The error is based on a replicate of five sets of data.

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

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

2.3 Bacteria inactivation using fluorescent light irradiation

Photocatalytic inactivation was carried out at six irradiation durations of 0, 15, 30, 45, 60 and 120 minutes. Triplicate measurements were taken for each experiment. The temperature and the relative humidity in the black-box were measured before and after individual experiments and the values were constant throughout the experiment. After irradiation, membrane filters were immediately removed from the Petri dishes and placed face-down on agar plates. For *E. coli* K-12 and *Pseudomonas fluorescens*, eosin methylene blue (EMB) agar plates were used, while tryptic soy agar (TSA) was used for *Microbacterium* sp., *Paenibacillus* sp. SAFN-007 and *Bacillus subtilis* and R2A agar was used for *Microbacteriaceae* str. W7. All the plates were sealed with parafilm tapes and placed in an incubator at 26^0C and 37^0C for *Pseudomonas fluorescens* and the other bacterial strains, respectively. The colonies were then counted using a colony counter daily on days 2 through 5, and on the 10th day, during which they were regularly checked for any regrowth of the colonies. 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. Since after 5 days, no new bacterial colony was observed, suggesting that the revival of bacteria exposed to heterogeneous photocatalytic inactivation after 5 days in dark was insignificant and could 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 1st order kinetics with respect to bacterial colony count (N_t), which is shown by Equation (1),

$$\ln (N_t/N_0) = - kt \quad (1)$$

Here, N_t = 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 = 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.

3.0 Results and Discussion

3.1 Bacteria inactivation under fluorescent light irradiation without TiO_2

In the control experiments carried in absence of fluorescent light, the six immobilized bacterial strains were exposed to 1116 mg/m^2 of TiO_2 to a dark environment up to two hours. It was found that the average colony counts for all the bacterial strains varied insignificantly with little deviation (standard deviation of 0-10 %). Hence, in the absence of fluorescent light, bacteria impregnated on a membrane surface seemed to be insensitive to TiO_2. The control experiments carried in absence of TiO_2, showed that the fluorescent irradiation alone inactivated various strains of bacteria, results of which are shown in Figure 2. Figure 2 shows that in two hours, around 40-50% of *E. coli* K-12, *Pseudomonas fluorescens* and *Paenibacillus* sp. SAFN-007 were inactivated. Although *Microbacteriaceae* str. W7 showed negligible inactivation (not shown in Fig. 2), 20% of *Bacillus subtilis* and only 13% of *Microbacterium* sp. were inactivated. The observed inactivation in the absence of TiO_2 shown 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 oxygen radicals within the cells, which cause oxidative stress and lead to cell damage [11]. In addition, long wavelength UV light (i.e., 320-400 nm) has been reported to mainly damage organisms by exciting photosensitive molecules within the cell, thus producing active species, such as $O_2^{\cdot}$, H_2O_2, and $^{\cdot}OH$, to adversely affect the genome and other intracellular molecules sublethally or lethally causing cell mutations, growth delay, etc. [17].

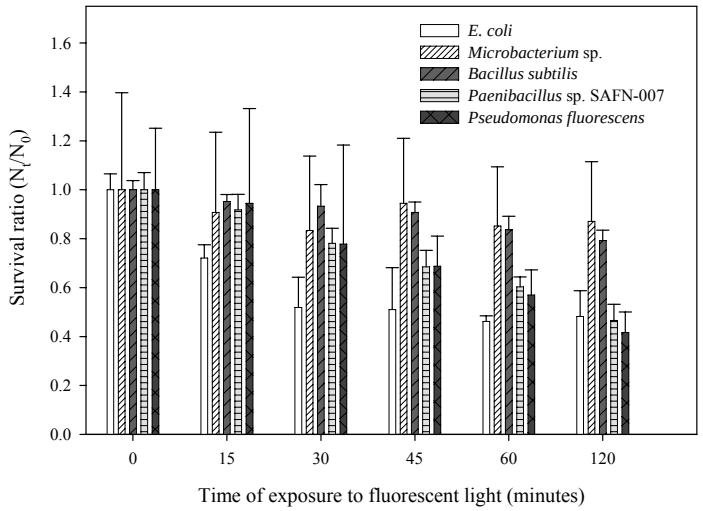

Figure 2. Survival ratios (N_t/N_0) as a function of exposure duration (without TiO_2) for *E. coli* K-12, *Pseudomonas fluorescens*, *Microbacterium* sp., *Paenibacillus* sp. SAFN-007 and *Bacillus subtilis*. The data shown in this figure are the averages of three replicates. Here, N_t = the number of CFUs after irradiation for t minute and N_0 = the number of CFUs at 0 minute.

3.2 Bacteria inactivation under fluorescent light irradiation and TiO_2

The inactivation of bacteria in the presence of TiO_2 irradiated by fluorescent light exhibited first order reaction kinetics and the rate of inactivation increased with increased exposure to fluorescent light for all the bacteria. However, the variation in the inactivation rate constant with an increase in the TiO_2 loading was different for the different bacteria. Figure 3 shows the inactivation rate constants of the six bacterial strains at different TiO_2 loadings. Gram-negative bacteria *E. coli* K-12 and *Pseudomonas fluorescens* showed the highest inactivation rate (0.0078-0.2442 min^{-1}) while the Gram-positive bacteria appeared to be more resistant to the photocatalytic inactivation (Figure 3). This 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, such as *S. aureus*, *E. faecium* [11], and *L. helveticus* [12]. Although the exact mechanism of photocatalytic inactivation of bacteria remains unclear, the thicker cell wall of Gram-positive bacteria could better protect them from ROS attack than Gram-negative bacteria. Gram-positive bacteria have a complex cell wall structure with plasma membranes surrounded by a 30-Å thick peptidoglycan wall, which is further covered by an 80 Å outer membrane, consisting of a mosaic of proteins, lipids and lipopolysaccharides [13], whereas Gram-negative bacteria contain a typical cell-wall thickness of ~250 Å, composed of peptidoglycan and techoic acid. Hence, during heterogeneous photocatalysis, a thicker cell wall likely results in lower inactivation. Table 2 (1-f) shows the variation of the survival ratios with respect to irradiation time and TiO_2 loadings for the six bacterial strains.

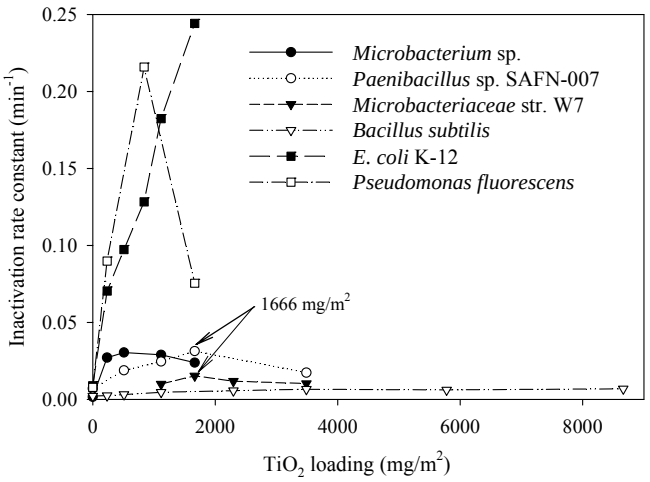

Figure 3. Inactivation rate constant vs. the TiO_2 loading for Gram-negative and Gram-positive bacteria.

Table 2. Survival ratios (N_t/N_0) and errors with respect to irradiation time (min) and TiO_2 loading (mg/m^2) for (a) *Microbacterium* sp., (b) *Paenibacillus* sp. SAFN-007, (c) *Microbacteriaceae* str. W7, (d) *Bacillus subtilis* (e) *Pseudomonas fluorescens* and (f) *E. coli*.

(a)

Time (min)	TiO_2 loading									
	234 (mg/m^2)		511 (mg/m^2)		840 (mg/m^2)		1116 (mg/m^2)		1666 (mg/m^2)	
	N_t/N_0	Error	N_t/N_0	Error	N_t/N_0	Error	N_t/N_0	Error	N_t/N_0	Error
0	1.0	0.13	1.0	0.00	1.0	0.40	1.0	0.63	1.0	0.00
15	0.80	0.14	0.48	0.13	0.93	0.36	0.44	0.23	0.89	0.12
30	0.76	0.17	0.48	0.06	0.74	0.30	0.71	0.35	0.80	0.24
45	0.55	0.14	0.49	0.06	0.91	0.33	0.18	0.08	0.61	0.13
60	0.33	0.04	0.30	0.16	0.47	0.16	0.11	0.09	0.20	0.15
120	0.00	0.00	0.01	0.01	0.07	0.09	0.02	0.01	0.04	0.03

(b)

Time (min)	TiO_2 loading							
	511 (mg/m^2)		1116 (mg/m^2)		1666 (mg/m^2)		3490 (mg/m^2)	
	N_t/N_0	Error	N_t/N_0	Error	N_t/N_0	Error	N_t/N_0	Error
0	1.0	0.12	1.0	0.07	1.0	0.11	1.0	0.12
15	0.86	0.12	0.60	0.05	0.69	0.08	0.64	0.09

30	0.62	0.05	0.44	0.12	0.52	0.09	0.47	0.06
45	0.40	0.08	0.27	0.08	0.31	0.04	0.41	0.04
60	0.24	0.10	0.18	0.06	0.17	0.05	0.29	0.06
120	0.12	0.06	0.07	0.04	0.02	0.03	0.16	0.02

(c)

Time (min)	TiO_2 loading							
	1116 (mg/m^2)		1666 (mg/m^2)		2297 (mg/m^2)		3490 (mg/m^2)	
	N_t/N_0	Error	N_t/N_0	Error	N_t/N_0	Error	N_t/N_0	Error
0	1.0	0.26	1.0	0.25	1.0	0.12	1.0	0.0
15	0.86	0.24	0.83	0.23	0.76	0.09	0.94	0.17
30	0.77	0.16	0.66	0.14	0.72	0.06	0.79	0.12
45	0.67	0.15	0.51	0.14	0.59	0.08	0.64	0.03
60	0.51	0.20	0.38	0.24	0.50	0.07	0.50	0.24
120	0.01	0.01	0.02	0.01	0.03	0.00	0.01	0.00

(d)

Time (min)	TiO_2 loading											
	234 (mg/m^2)		511 (mg/m^2)		1116 (mg/m^2)		2297 (mg/m^2)		3490 (mg/m^2)		5778 (mg/m^2)	
	N_t/N_0	Error	N_t/N_0	Error	N_t/N_0	Error	N_t/N_0	Error	N_t/N_0	Error	N_t/N_0	Error
0	1.0	0.105	1.0	0.04	1.0	0.10	1.0	0.10	1.0	0.08	1.0	0.03
15	0.92	0.09	0.88	0.04	0.87	0.10	0.76	0.06	0.896	0.09	0.94	0.03
30	0.88	0.07	0.85	0.04	0.73	0.06	0.77	0.12	0.73	0.08	0.90	0.09
45	0.86	0.07	0.83	0.03	0.71	0.08	0.70	0.10	0.67	0.06	0.77	0.08
60	0.80	0.06	0.80	0.03	0.70	0.07	0.68	0.09	0.64	0.05	0.57	0.04
120	0.76	0.07	0.69	0.13	0.66	0.06	0.57	0.07	0.50	0.05	0.5	0.04

(e)

Time (min)	TiO_2 loading					
	234 (mg/m^2)		840 (mg/m^2)		1666 (mg/m^2)	
	N_t/N_0	Error	N_t/N_0	Error	N_t/N_0	Error
0	1.0	0.51	1.0	0.14	1.0	0.25
15	0.17	0.09	0.04	0.05	0.38	0.13
30	0.06	0.11	0.00	0.00	0.10	0.08
45	0.02	0.01	0.00	0.00	0.00	0.00
60	0.00	0.00	0.00	0.00	0.00	0.00
120	0.00	0.00	0.00	0.00	0.00	0.00

(f)

Time (min)	TiO_2 loading									
	234 (mg/m^2)		511 (mg/m^2)		840 (mg/m^2)		1116 (mg/m^2)		1666 (mg/m^2)	
	N_t/N_0	Error	N_t/N_0	Error	N_t/N_0	Error	N_t/N_0	Error	N_t/N_0	Error
0	1.0	0.70	1.0	0.24	1.0	0.16	1.0	0.48	1.0	0.02
15	0.44	0.29	0.11	0.08	0.12	0.04	0.04	0.04	0.03	0.03
30	0.07	0.05	0.01	0.02	0.02	0.04	0.01	0.01	0	0
45	0.07	0.05	0.01	0.02	0	0	0	0	0	0
60	0.01	0.01	0.01	0.00	0	0	0	0	0	0
120	0.0	0.0	0	0	0	0	0	0	0	0

* Error = $(N_t/N_0)\times(((a/N_t)\text{^}2 + (b/N_0)\text{^}2)\text{^}0.5)$, where N_t and N_0 are experimental variables whose standard deviations are a and b, respectively.

In case of *E. coli* K-12, under a high TiO_2 loading of 1666 mg/m^2, over 96% of the bacteria were inactivated within 15 minutes of exposure, and all the bacteria were inactivated after a 30-minute or longer exposure (Table 2-f). 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 with that reported by Huang et al., 2000 [21] who reported the damage of cell walls of *E. coli* within 20 minutes of exposure to UV-A light at 0.8 mW/cm^2 with the presence of TiO_2. Since a higher TiO_2 loading may enhance the generation of reactive oxygen species (ROS), causing damage to the cell wall, the cytoplasmic membrane, and other intracellular components, the resultant inactivation rate was substantially increased. This is consistent with previous studies that higher inactivation of *E. coli* was observed when higher TiO_2 concentrations were adopted with UV-visible radiation longer than 380 nm [18], or under UV-A irradiation [19].

The inactivation rate constant of Gram-positive bacteria *Paenibacillus* sp. SAFN-007 and *Microbacteriaceae* str. W7 reached a maximum at a TiO_2 loading of 1666 mg/m^2 (Figure 3). This loading corresponds to a 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 incompletely absorb light of 365 nm [20],

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 because increases in the TiO_2 concentration can cause terminal reactions (shown as reactions (3) and (4) below), which can form less reactive hydroperoxyl radicals ($HO_2\cdot$) and decrease the bacterial inactivation efficiency [10].

$$\cdot OH + \cdot OH \rightarrow H_2O_2 \quad (3)$$
$$H_2O_2 + \cdot OH \rightarrow H_2O + HO_2\cdot \quad (4)$$

Interestingly, unlike other Gram-positive bacteria shown in Table 2, the inactivation rate constant of *Bacillus subtilis* reached a plateau at a loading higher than 2297 mg/m^2 (Figure 3). The stronger resistance of *Bacillus subtilis* to inactivatioin could be due to its transformation into endospores and becoming insensitive to the changes in the environment (e.g., increases in the TiO_2 loading). Although in the absence of TiO_2, two hours of exposure to fluorescent light inactivated ~ 21% of the *Bacillus subtilis* (Figure 2), 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 *Bacillus subtilis* were little affected by either a small amount of UV-A irradiation or increased TiO_2 loading. This can be supported by Kuhn et al. [11], who reported that the spores of *Bacillus subtilis* were well resistant to 60 minutes of UV-A photocatalytic treatment. For *Microbacterium* sp. and *Microbacteriaceae* str. W7, one $\log_{10}$ inactivation was obtained after 2 hours of light exposure at all TiO_2 loadings, while for *Paenibacillus* sp. SAFN-007, the same inactivation occurred in the TiO_2 loading range of 1116-1666 mg/m^2, while 100% inactivation of *Pseudomonas fluorescens* was obtained after 45 minutes of exposure to light at a TiO_2 loading of 840-1666 mg/m^2 (Table 2 a-e).

Limited experiments were carried out using another TiO_2 photocatalyst, Hombikat UV-100 for Gram-positive bacterium *Paenibacillus* sp. SAFN-007, at the TiO_2 loadings of 234, 511, 1116 and 1666 mg/m^2. Hombikat UV-100 is less active than Degussa P25 and the difference is more significant at higher TiO_2 loading. At the TiO_2 loading larger than 1116 (mg/m^2), the photocatalytic activity of Degussa P25 is 1.83-5.41 times higher than that of Hombikat UV-100. Rincon and Pulgarin (2003)[10] reported that a mixture of anatase and rutile showed better photocatalytic activity than anatase or rutile alone. Since Degussa P25 is a mixture of 80% anatase and 20% rutile, it is not surprising that Degussa P25 exhibited a higher inactivation efficiency than Hombikat UV-100 (100% anatase).

4.0 Conclusion

The results show that 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 *E. coli* was the most effective and *Bacillus subtilis* was the least effective in photocatalytic inactivation. Of the six strains of bacteria studied, four showed maximum inactivation at an optimum TiO_2 loading in the range of 511-1666 mg/m^2, corresponding to a thickness of 294 - 438 nm of TiO_2 on the surface. Complete inactivation of *E. coli* was achieved after 30 minutes of exposure to

fluorescent light at TiO_2 loading of 1666 mg/m^2 (438 nm of TiO_2 coating). This thickness of TiO_2 can be applied on indoor wall commonly illuminated with fluorescent lighting to induce sufficient inactivation of the indoor bacteria. The study also indicates that reaction with OH radical and reactive oxygen species is less significant for Gram-positive bacteria in comparison to Gram-negative bacteria.

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