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RESEARCH PAPER

Efficacy of Photocatalytic HEPA Filter on Reducing Bacteria and Fungi Spores in the Presence of UVC and UVA Lights

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ABSTRACT

The Indoor Air Quality (IAQ) of a hospital is very important to properly protect both patients and the staff against hospital infections. The present study aims at evaluating the efficiency of photocatalytic filters as well as the impact of important factors such as the type of UV wavelength (UVC, UVA) with different intensities and loading rates of TiO_2 in HEPA Filters on reducing airborne microorganisms. For so doing, it has prepared photocatalytic filters by dipping them into 2% and 4% titanium dioxide suspensions as low and high loading, respectively. The experiments have been carried out on four species' microorganisms, namely Epidermidis, Subtilis, Niger, and Penicillium. Fungi and bacteria suspensions have been prepared with concentrations of 10^6, 10^7 CFU/m^3, respectively. In terms of microorganism removal, the efficiency of HEPA filters in both types of TiO_2 loading and UVC and UVA radiations with two intensities at three times intervals (60, 90, and 120 min) have been investigated. Results show that lower penetration microorganism belong to PCO (TiO_2 + UV), compared to photolysis (UV alone) at all intervals of UV radiation. TiO_2 loading has no significant effect on percentage removal in all microorganisms. The percentage penetration of microorganisms under UVC radiation is lower than UVA radiation. Also, increasing the radiation intensity in both types of UV shows that it has higher effectiveness for removing bacteria and fungi. Therefore, the use of photocatalytic HEPA filters with UVC radiation can play an influential role in reduction of the microorganisms in different places such as hospitals, cleanrooms, etc.

KEYWORDS: Photocatalytic oxidation; photolysis; Airborne microorganisms; High efficiency particulate air Filter; UV radiation

INTRODUCTION

Airborne microorganisms are one of the significant sources of indoor air pollution in hospitals (Pal et al., 2007). Bioaerosols containing viruses, bacteria, and fungi can be responsible for

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infectious diseases, toxic reactions, and allergic responses (Zacarías et al., 2015). The control of indoor air quality of hospitals plays an important role in the prevention and protection of both hospital staff and patients from hospital infection. Improper ventilation may contribute to poor indoor air quality of Hospitals and it was caused Hospital infection and occupational diseases(Leung and Chan, 2006).

In general, there are three methods for removing airborne microorganisms from the indoor environment namely, such as source control, increase ventilation and air cleaning, of which applying air cleaning equipment such as the high-efficiency particulate air filter is one of the most important methods of improving indoor air quality(Ao and Lee, 2005). Recently, Photocatalytic oxidation (PCO) has shown to be an effective technology for eliminating bacteria and fungi; it involves the use of UV radiation onto a photocatalyst such as TiO_2 (Benabbou et al., 2007; Rengasamy et al., 2004). When a photocatalyst, mainly TiO_2 is illuminated with UV light, the electrons in the photocatalyst will be transferred from the valence band to the conduction band, thus electron (e^-) and hole (h^+) pairs will be created on its surface. The electrons in the conduction band react with oxygen and yield superoxide radicals, O_2^-, while the positive holes react with hydroxide, mainly from water, and generate hydroxyl radicals(Zhang et al., 2006; Keller et al., 2005; Oguma et al., 2002). These free radicals have high oxidizing properties and are capable of attacking the cell membranes of microorganisms, which ultimately destroy microorganisms(Yang and Wang, 2008).

Today, TiO_2 is widely used as a photocatalyst because of its superior characteristics. It is inexpensive, very stable, showing high photocatalytic efficiency and safe towards both humans and the environment(Gupta and Tripathi, 2011). The photocatalyst (TiO_2) by generating radicals can degrade a broad range of contaminants and organic compounds and turn into CO_2 and H_2O (Zhao and Yang, 2003). Furthermore, titanium dioxide under UV light leads to the oxidation and decomposition of many types of bacteria, organic and inorganic materials. Titanium dioxide exists in three crystalline modifications: rutile, anatase, and brookite. Rutile phase is the most abundant phase in the earth. Brookite phase is rare and does not exhibit any photocatalytic properties. Compared with rutile and brookite, anatase shows the highest photoactivity(Stamate and Lazar, 2007). The energy band-gaps of anatase and rutile are 3.23 and 3.02 eV, respectively(Mo et al., 2009). Anatase is superior to rutile; as opposed to the case in rutile, in the anatase very stable surface peroxide groups can be formed during photo-oxidation reaction and also, anatase is more suitable for driving conjugate reactions involving electrons. Therefore, it will be more capable of eliminating contaminants(Zhao and Yang, 2003; Vequizo et al., 2017).

A great deal of research has already been published on the use of photocatalytic oxidation to eliminate volatile organic compounds(Huang et al., 2016; Zuo et al., 2006). Recent studies focused on using PCO to disinfect microorganisms in water and soil(Bodzek and Rajca, 2012; Le et al., 2012). Furthermore, numerous studies on photocatalytic inactivation of different types of microorganisms in air have been reported, which most studies on the use of TiO_2 for photocatalytic disinfection has mainly focused on membrane filters, fabric filters and glass filters, and studies on the inactivation of microorganisms, such as bacterial and fungal spores with the use of high-efficiency particulate air filter (HEPA) are less frequent. Few studies were conducted on photocatalytic HEPA filters and their experimental results showed a significant increase in the efficiency of removal of microorganisms(Chotigawin et al., 2010; Chuaybamroong et al., 2010). Therefore, we tried to produce a photocatalytic HEPA filters by loading TiO_2 to investigate its performance for removal bacteria and fungi.

The effects of UV wavelength and their intensity levels which are important parameters to evaluate the efficiency of photocatalytic filters were not investigated in photocatalytic HEPA filters. For the first time in this study two types of UV radiation (UVC, UVA) at two intensity levels on the efficiency of photocatalytic HEPA filters in closed loop chamber under certain temperature and humidity was investigated, in order to removal bacteria and fungi.

MATERIALS AND METHODS

In order to measure the performance of the filters and to determine the environmental conditions, closed-loop chamber was designed and manufactured with dimensions 170 cm long, 60 cm wide and 20 cm high using galvanized sheets (Chotigawin et al., 2010). The equipment required for completing the closed-loop chamber was installed as Fig.1 at designated locations(Mousavi et al., 2017).

Mini pleated HEPA filter with size 20×20 cm (99.99% ASHRAE efficiency, class H_{14} (Camfil., Sweden)) were washed in a solution of 0.08 M sodium dodecyl sulfate in order to remove water- resistance. filters were coated by dipping into 2% and 4% TiO_2 suspensions, and then dried at room temperature for 24 hours. At the end, the filters were baked in an oven at 120°C for 1 hour(Chotigawin et al., 2010). Photocatalytic filters with two TiO_2 loading were investigated: low loading (1984±104 mg/m^2) and high loading (3967± 89 mg/m^2). The pressure drop of the test filter was measured with a digital differential manometer (Microprocessor, AIR FLOW., England). TiO_2 was tested by X-ray diffraction to discriminate the percentage of TiO_2 phase (Diffractometer., Model Equinox 3000, France). morphology of coated and uncoated HEPA filter was observed by Scanning Electron Microscopy (SEM, Model 30 Philips-XL., USA).

All microorganisms were purchased from the Industrial Research Institute of Iran. S.epidermidis was cultured on tryptic soy agar (TSA) at 37°C for 24 hours while B. subtilis was cultured at 37 °C for 7 days. The spores were harvested into sterile distilled water, heated at 80°C for 10 minutes, and centrifuged at 2,500 rpm for 5 min to inactivate vegetative cell. P. citrinum was incubated at 25°C for 7 days into sterile distilled water and centrifuged at 2,000 rpm for 5 min. A. niger was inoculated on Sabouraud Dextrose Agar and grown at 37°C for 3 days. Finally, suspension of bacteria and fungi was prepared at concentrations of 10^7 and 10^6 CFU/ml, respectively by sterile distilled water(Lin and Li, 2003; Vohra et al., 2006).

In order to determine the efficiency of uncoated and coated filters, each of the filters was installed inside the closed-loop chamber. The bacteria and fungi suspensions were separately sprayed into the closed-loop chamber by a nebulizer (OMRON., Japan) at 60, 90 and 120 min, at a pressure of 15-20 psi. The blower drew the air to circulate in the closed-loop chamber at a velocity of 0.3 m/s measured with a hot wire anemometer (Model 1340, TES., Taiwan). At this time, UVC lamps (Model TUV 6W G6 T5, PHILIPS., POLAND) were turned on and the experiment was investigated with no radiation. The temperature and the relative humidity were 25 °C and 35±5%, respectively. A Quick Take sampler (Model 30, SKC., USA) was used containing agar culture plate at a sampling flow rate of 28.3 liters per minute. For determining the penetration of airborne microorganisms from the filters, air sampling before and after of test filter was done. In order to determine colonies growth, the sampled plates were incubated at 20-25 °C for 3-5 days for fungi and at 37 °C for 24-48 hours for bacteria. After counting the colonies that penetrated from the filters by the colony counter, the amount of microorganism penetration was calculated by difference between the number of upstream and downstream colonies of the filter. The result of the efficiency of the uncoated filter was compared with photocatalytic filters, with low and high loading in UVC radiation and dark mode.

The effect of two types of UV wavelengths (UVA 360 nm and UVC 254 nm) with low and high intensity on the efficiency of photocatalytic filters was evaluated. The bacteria and fungi suspensions were separately sprayed into the channel by a nebulizer for 30 min. UVC lamps (Model TUV 6W G6 T5, PHILIPS., POLAND) and UVA lamps (Model F6T5.6 W, Hitachi., Tokyo, Japan) with irradiance of low (mw/cm^2 1.4±0.09) and high (mw/cm^2 2.2±0.06) intensity were turned on. Irradiance intensity was measured with a UV-C meter (Model 254, Lutron., Taiwan) and UV-A meter (Model EC 1, Hanger., Sweden) on the filter surface. Data were analyzed by t-test and the analysis of variance (ANOVA) was conducted at a confidence level of 95%, using SPSS software version 23.

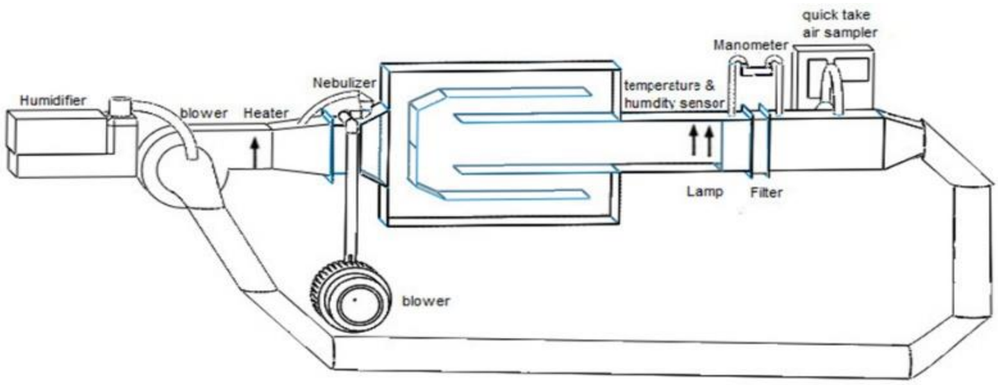


Figure 1. Schematic of the closed-loop chamber

RESULTS AND DISCUSSION

Morphology characterization of TiO_2

According to the results of the XRD test, 70% TiO_2 was detected in the anatase phase and about 30% was detected in the rutile phase (Fig.2). Previous research has indicated that anatase band gap is higher than rutile(Zhang et al., 2014). Therefore, anatase has the largest share of photocatalytic activity and is more capable of eliminating toxic and dangerous pollutants as well as degradation of microorganisms. As a result, TiO_2 was appropriate for coating on HEPA filters. The pressure drop in the uncoated filter, photocatalytic filters with low and high loadings was measured as 45, 67 and 73 Pa, respectively.

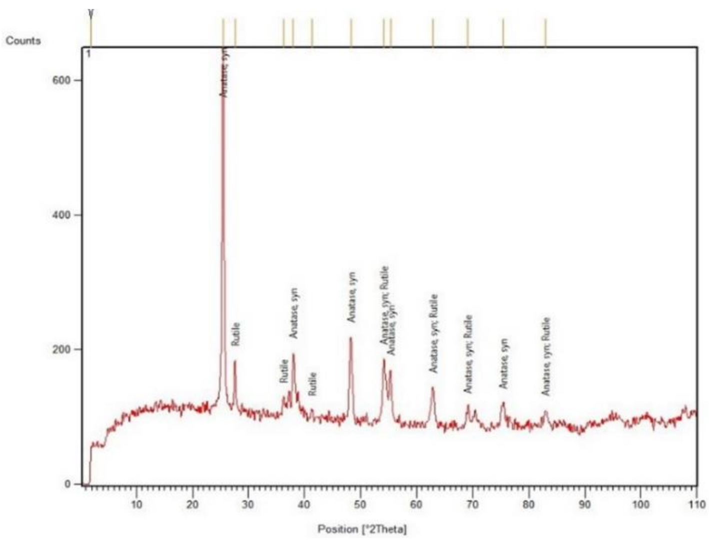
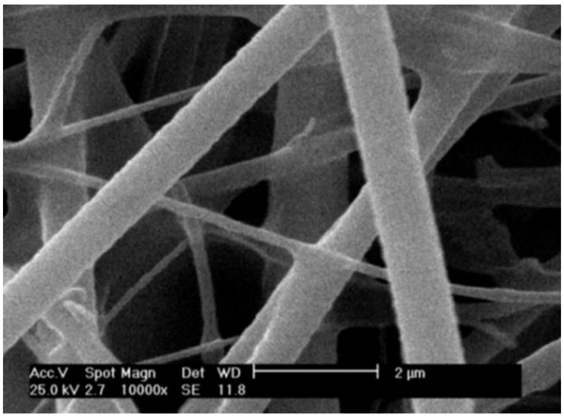
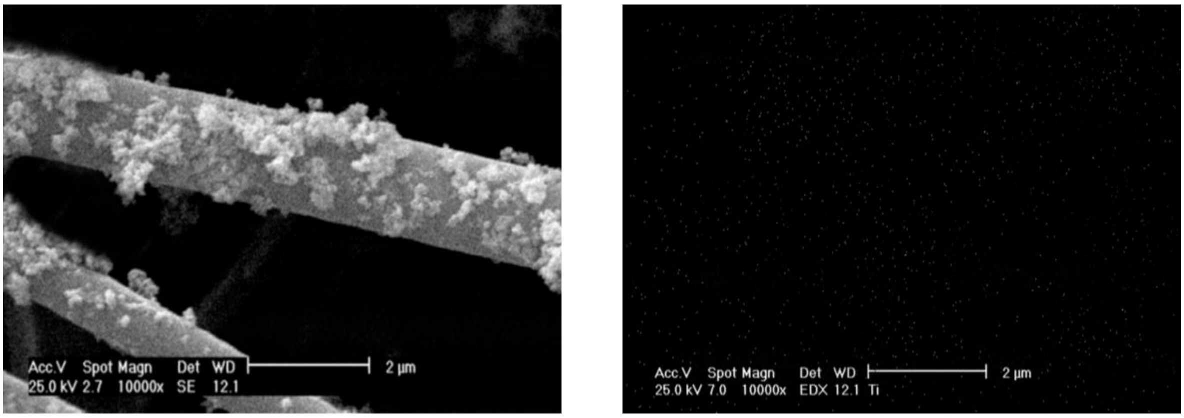


Figure 2. The TiO_2 analysis graph by X-ray diffractometer

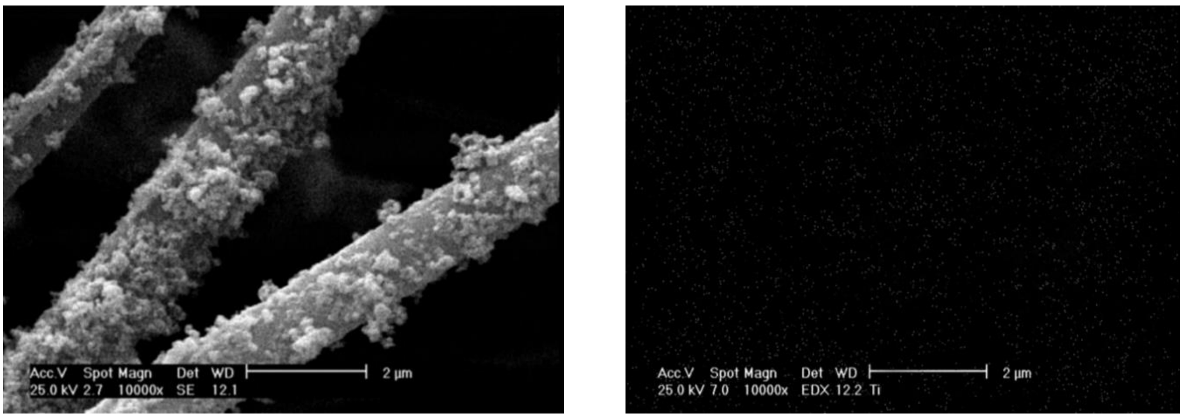
Fig. 3 shows the morphology of fibers before and after coating with two TiO_2 loadings as observed by SEM and elemental map of TiO_2 particles. The white points indicate presence of TiO_2 particles in the HEPA filter. Elemental map of High loading of titanium dioxide (3967±104 mg/m^2) showed that it coated with a large amount of TiO_2 particles and it had high density on the filter fiber than the low loading (1984±89 mg/m^2). High density of TiO_2 can cause the increased filter resistance, decreased air permeability (Bao et al., 2016).



a



b



c

Figure 3. Morphology of fibers (10,000× magnifications for all micrographs), neat HEPA filter (a), ones with low TiO_2 loading (b) and high TiO_2 loading (c)

Effect coated and uncoated filters

Table.1. shows the airborne microorganism penetration from the uncoated filter and also photocatalytic filters. The results of microorganism penetrations from uncoated filter showed a significant difference between light and dark mode in three intervals of 60, 90 and 120 minutes. Also, the penetration rate of all four types of microorganism from photocatalytic filters with low and high loadings in three times intervals 60, 90 and 120 min in UVC irradiation was reduced compared to the dark mode ($P_{<}$ 0.001). In the dark section, the difference between coated and uncoated filters was insignificant ($P > 0.05$). The finding showed that the percentage penetration of all microorganism was decrease by increasing the duration of UVC radiation to the HEPA filter. Lower penetration for all airborne microorganism was also found from the PCO (TiO_2 + UVC) than UVC radiation alone. Moreover, the results showed that the microorganism penetration from photocatalytic filters ($P_{<}$ 0.001) was reduced more than the uncoated filter when all were irradiated ($P_{=}$ 0.02- 0.04). Because TiO_2 under UVC radiation generate superoxide and hydroxyl radicals, these radicals will effectively eliminate microorganisms.

Table 1. Efficacy of coated and uncoated filters on S. epidermidis penetration(a), B. subtilis penetration(b), A. niger penetration (c), P. citrinum penetration (d)

Penetration(%)	S.epidemidis					
Tio2 loading / Time(min)	Low loading		High loading		uncoated	
	dark	UVC	dark	UVC	dark	UVC
60	74%	5%	63%	4%	92%	55%
90	67%	6%	56%	5%	79%	50%
120	41%	5%	31%	3%	58%	29%

a

Penetration(%)	B.subtilis					
Tio2 loading / Time(min)	Low loading		High loading		uncoated	
	dark	UVC	dark	UVC	dark	UVC
60	33%	6%	32%	4%	92%	30%
90	17%	5%	16%	3%	81%	13%
120	14%	4%	11%	2%	50%	10%

b

Penetration(%)	A.niger					
Tio2 loading / Time(min)	Low loading		High loading		uncoated	
	dark	UVC	dark	UVC	dark	UVC
60	40%	20%	36%	18%	90%	33%
90	35%	15%	34%	12%	78%	28%
120	33%	6%	32%	5%	64%	24%

c

Penetration(%)	P.citrinum					
Tio2 loading / Time(min)	Low loading		High loading		uncoated	
	dark	UVC	dark	UVC	dark	UVC
60	50%	34%	43%	30%	95%	41%
90	39%	17%	35%	16%	87%	33%
120	34%	5%	30%	3%	61%	28%

d

Effect the TiO_2 loading

There was no statistically significant difference in the reduction of microorganisms in the two types of loading ($P_{=}$ 0.182 for S. epidermidis, $P_{=}$ 0.111 for B.subtilis ,$P_{=}$ 0.283 for A.niger , $P_{=}$ 0.277 for P.citrinum) (Table.2). Since the loading rate has little effect on the efficiency of photocatalytic filters, only photocatalytic filter with low loading was investigated to assess the effect of UV wavelength (UVC, UVA) at two intensity levels.

Table 2. Efficacy of low and high loading TiO_2 on bacteria penetration (a) and fungi penetration (b)

Penetration(%)	S.epidemidis				B.subtilis			
Tio_2 loading / Time(min)	Low loading		High loading		Low loading		High loading	
	dark	UVC	dark	UVC	dark	UVC	dark	UVC
60	74%	5%	63%	4%	33%	6%	32%	4%
90	67%	6%	56%	5%	17%	5%	16%	3%
120	41%	5%	31%	3%	14%	4%	11%	2%

a

Penetration(%)	A.niger				P.citrinum			
Tio_2 loading / Time(min)	Low loading		High loading		Low loading		High loading	
	dark	UVC	dark	UVC	dark	UVC	dark	UVC
60	40%	20%	36%	18%	50%	34%	43%	30%
90	35%	15%	34%	12%	39%	17%	35%	16%
120	33%	6%	32%	5%	34%	5%	30%	3%

b

Table 3 shows The low intensity of UVC radiation (1.4±0.09 mw/cm^2) had better efficacy in reduction of all four types of S. epidermidis, B. subtilis, A. niger and P. citrinum microorganisms than the low intensity of UVA radiation ($P_{<}$ 0.001). Also, more reduction of airborne microorganisms was observed in the high intensity of UVC radiation (2.2±0.06 mw/cm^2) compared to the UVA ($P_{<}$ 0.001). As a result, UVC radiation to the surface of photocatalytic filters in both low and high intensity was more effective in reducing microorganisms than UVA radiation. Because UVC wavelength is shorter (higher energy) than UVA, therefore, UVC has the higher energy to stimulate TiO_2 electrons. In the ultra-high intensity of UVC radiation compared to the low intensity, more reduction of airborne microorganisms was observed ($P_{=}$ 0.038). Also, in high intensity of UVA radiation, the concentration of the studied microorganisms decreased significantly compared to low intensity ($P_{=}$ 0.045). When irradiance intensity was increased, significant increases in reducing microorganism penetration was observed.

Table 3. Comparative effectiveness of UV wavelengths in low and high intensity on microorganism penetration

	UVC		UVA	
Microorganisms Penetration(%)	low	high	low	high
S.epidemidis	40%	30%	60%	45%
B.subtilis	22%	16%	44%	30%
A.niger	19%	13%	33%	27%
P.citrinum	29%	20%	43%	33%

In this study, it was found that radiation UVC to the uncoated surfaces and also surfaces loaded with Titanium dioxide reduced microorganisms similar to the study by Thi Tuyet Nhung et al. (2012) that obtained inactivation of Bacillus with UVC radiation in both uncoated and coated films(Thi Tuyet Nhung et al., 2012). The study by Kuehn et al (2003) showed that 80% disinfection of S. epidermidis from UVA radiation alone may be the result of the oxidative degradation of microorganisms(Kuehn, 2003). Cadet (2005) conducted a study titled "Ultraviolet radiation-mediated damage to Cellular DNA" and stated that the quantitative and qualitative damaging of microorganism is dependent on the nature of the cells and amount of photosensitizers(Cadet et al., 2005). Concerning UVA photolysis reaction, UVA was effective only on bacteria disinfection, but not effective on fungi(Chotigawin et al., 2010), the present finding showed that UVC radiation was effective on both bacteria and fungi spores since UVA is effective in degradation of vegetative cells due to low energy intensity, while UVC has a significant effect on the destruction of spores due to increased energy intensity(Thi Tuyet Nhung et al., 2012). Therefore, in this study, UVC radiation had a considerable effect on the reduction of A. niger and P. citrinum spores.

The UVC radiation to the surface of photocatalytic HEPA filters in both low and high loading had an increasing effect on reducing microorganisms as compared to photolysis ($P<0.001$). Nanoparticles of titanium dioxide produced radicals' superoxide and hydroxyl after exposure to UVC radiation which was the cause of the destruction of microorganisms on the surface of the filter. Therefore, it can be said that the photocatalytic oxidation (TiO_2 + UVC) has a significant effect on the reduction of penetration of microorganism compared to the use of UVC alone (photolysis). The study of Chotigawin et al. showed that PCO was more successful for disinfection of microorganisms than UVA photolysis alone(Chotigawin et al., 2010). According to Greist et al (2002) study, when spores are exposed to PCO, their hydrophobic exteriors are mineralized and external changes of spores occur, producing radicals that allow the hydrophilic interiors to interact with the photocatalyst inactivation of cells(Greist et al., 2002).

In the present study, there were no statistically significant differences between the two TiO_2 loading in both radiations and the no radiation of UVC ($P > 0.005$). This also agrees with the study of Chotigawin et al. (2010) who observed no significant difference in the rate of destruction of microorganisms in UVA radiation between low and high TiO_2 loading. They found that epidermidis was completely destroyed under low TiO_2 and high TiO_2 loading coated filter within 4 hours and 2 hours, respectively(Chotigawin et al., 2010). Therefore, it can be concluded that HEPA filter with high loading of TiO_2 has a better effect on reducing the degradation time of S. epidermidis compared to the low loading, but these two loadings of photocatalytic filters statistically show the same result in the destruction of microorganisms. Pal et al demonstrated that Increasing TiO_2 loading on acetate membrane filter up to 2300 mg/m^2 could increase inactivation of B. subtilis, but loading higher than 2300 mg/m^2 could decrease disinfection efficiency(Pal et al., 2007). A possibility is that UVA has a limited depth of penetration. Hence, a thicker TiO_2 layer will not yield more radicals as only the surface layer can be exposed to UV light(Chuaybamroong et al., 2010). In this study, according to SEM images (Fig.3), it is observed that the TiO_2 layer loaded on the photocatalytic filter with high loading is much thicker than the lower loading, so the UVC radiation to the underlying layers is less, and lower radicals are generated compared to the surface layers. For this reason, there is no significant difference in the reduction of airborne microorganisms in both low and high loading of photocatalytic filters.

A higher penetration of microorganisms was observed Under UVA radiation regardless of UVC exposure. As a result, UVC radiation to the surface of photocatalytic filters is more effective in reducing microorganisms than UVA radiation. Because UVC wavelength is shorter than UVA, hence, UVC has the higher energy to stimulate TiO_2 electrons for the destruction of microorganisms. The low intensity of UVC radiation (1.4±0.09 mw/cm^2) has better efficacy in reduction of microorganisms than the low intensity of UVA radiation. Also, in the ultra-high intensity of UVC radiation compared to the low intensity, more reduction of airborne microorganisms was seen.

It was found that in high intensity UVA radiation, microorganism penetration decreased significantly compared to low intensity. Also, increasing UVC radiation from 1.4±0.09 mw/cm^2 to 2.2±0.06 mw/cm^2 could better reduce microorganisms. Chuaybamroong et al showed that irradiance intensity of UVA was increased from 0.85±0.18 to 4.85±0.09 mw/cm^2; insignificant increases in disinfection efficacy were observed. Besides, they reported that higher UVA irradiance lead to a competition between UVA and photocatalysis for dissolved O_2, which is needed for generating superoxide radicals. Because UVA is more effective in consuming O_2, photocatalytic inactivation is not enhanced although the irradiance intensity is much increased. Zhao et al. (2009) examined the effects of UVA intensity on B. subtilis. Their result showed that increased UVA intensities can generate superoxide radicals which can combine with hydroxyl radicals or holes at the TiO_2 surface, thereby reducing the microorganism inactivation effectiveness. Their results indicated that the maximum rate of photocatalytic inactivation was at 3 mw/cm^2, but beyond this intensity a negligible inactivation was observed(Zhao et al., 2009). As, in the present study, the UVA and UVC intensities were not beyond 3 mw/cm^2. Thus, increasing the intensity of UVA and UVC radiation showed better effectiveness in decreasing the penetration of microorganisms. Various factors affect in the performance of photocatalytic filters and the relative humidity is the main factor affecting the PCO reaction. Thus, it is better to evaluate the temperature, humidity and flow rate according to hospital air conditioners in different seasons of the year.

CONCLUSION

The results showed that lower penetration microorganism was found from PCO (TiO_2 + UV) compared to photolysis (UV alone) in three times UV radiation. Hence, the PCO can play an effective role in reducing microorganisms in hospital. Thus, coating HEPA filters with titanium dioxide under UVC radiation will increase the efficiency of HEPA filters in the reduction of airborne microorganisms. In the present study, according to the results, TiO_2 loading did not significantly effect on percentage removal in all of microorganism. The percentage penetration microorganism under UVC radiation was lower than UVA radiation. Also, increasing the radiation intensity in both types of UV showed higher effective on removal bacteria and fungi. In addition, the intensity of radiation and the type of UV radiation are also important factors affecting the efficiency of the photocatalytic filter.

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CONFLICT OF INTEREST

The authors declare that there is not any conflict of interests regarding the publication of this manuscript. In addition, the ethical issues, including plagiarism, informed consent, misconduct, data fabrication and/ or falsification, double publication and/or submission, and redundancy has been completely observed by the authors.

LIFE SCIENCE REPORTING

No life science threat was practiced in this research.

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