**Airborne Microorganism Disinfection by Photocatalytic HEPA Filter**

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**Abstract**

This study determined the efficacy of photocatalytic HEPA filters on microorganism disinfection in a closed-loop chamber and later applied it in an air purifier and tested its efficacy in an 8-m³ chamber and in a hospital. The photocatalytic filters were made by dip-coating a HEPA filter in a TiO₂ slurry. In order to disinfect the microorganisms retained on the filter, UV-A light was irradiated onto the filter to create strong oxidative radicals which can destroy microorganisms.

The findings showed that disinfection efficiency of the photocatalytic filters with high TiO₂ loading was insignificantly higher than with lower loading. *S. epidermidis* was completely eliminated within 2 hours, while 86.8% of *B. subtilis*, 77.1% of *A. niger*, and 82.7% of *P. citrinum* were destroyed within 10 hours. When applying the photocatalytic filters into an air purifier in a 8-m³ chamber, it was found that as soon as the air purifier was turned on, 83.4% of *S. epidermidis*, 81.4% of *B. subtilis*, 88.5% of *A. niger*, and 75.8% of *P. citrinum* were removed from the air. In a hospital environment, the PCO air purifier efficacy was lower than that in the chamber. Besides, relative humidity, distances from the air purifier and room size were suspected to affect the efficacy of the photocatalytic filters.

**Keywords:** high efficiency particulate air (HEPA) filter; photocatalytic oxidation; airborne microorganism; air purifier

1. Rationale and Background

Photocatalytic oxidation (PCO) is used for removing microorganisms; it involves the use of UV radiation onto a photocatalyst such as titanium dioxide (TiO₂). When it is illuminated, the electrons in photocatalyst will jump from the valence band to the conduction band, thus electron (e⁻) and hole (h⁺) pairs will be created on its surface. The electrons will combine with oxygen and become superoxide radicals, O₂⁻; while the positive holes will combine with hydroxide, mainly from water, and generate hydroxyl radicals. When organic compounds contact on the surface of the photocatalyst (TiO₂), these radicals will oxidize them and turn into carbon dioxide and water (Zhao and Yang, 2003). In case of microorganisms, the reactive oxygen species can damage nucleic acids of the microorganism, disturbing cellular functions, resulting in cell death (Yang and Wang, 2008).

Currently, there is much research applying the PCO onto different substrates for eliminating bacteria and fungi. For example, Griest et al., 2002 applied the PCO to aluminum disks to disinfect *Serratia marcescens* and found the most significant changes in bacteria cell morphology after 11.75 hours of exposure and more than 99% destruction acquired in 36 hours. For *B subtilis* and *A. niger* spores, the changes were observed after 11.75 hours and 36 hours, respectively. Lin and Li, 2003 applied the PCO to a glass slide and found the survival of *B. subtilis* and *P. citrinum* of 50% at 2.55 hours. Küehn et al., 2003 applied the PCO to a Plexiglas® substrate to destroy *E. coli*, *P. aeruginosa*, *S. aureus* and *E. faecium* and found the removal of more than 6log₁₀ in 60 minutes. Vohra et al., 2005 applied PCO to a fabric substrate in a recirculating chamber and found almost 76% of *B. subtilis* endospore destroyed in the first 4 hours. Pal et al., 2007 applied PCO to a membrane filter and found complete removal of *E. coli* within 30 minutes, while *Microbacterium sp.*. *Paenibacillus sp.*. SAFN-007 and *Microbacteriaceae* str.W7 were removed around 11log₁₀ after 2 hours.

However, the extensive literature on the use of TiO₂ for photocatalytic disinfection has mainly focused on membrane filters, fabric filters, and glass filters in a lab-scale, not yet to the high efficiency particulate air filter (HEPA). The application of photocatalytic HEPA filters for microorganism removal, in the form of an air purifier, is still scarce. One study has referred to using a HEPA filter as a substrate for PCO in air purification (Lesavoy and Peccia, 2006) but provided no scientific evidence on methodology, conditions and results. Another study applied the PCO to an activated...
carbon filter in an air cleaner for removing the VOCs, but not for bioaerosols (Ao and Li, 2005). Therefore, this study is a pioneer work using photocatalytic HEPA filter in an air purifier for microorganism disinfection. This work should provide a method for microorganism decontamination in indoor air, especially in tropical regions, where regular HEPA filters can be a breeding ground for microorganisms.

2. Materials and Methods

2.1. Filter immobilization

A 20 cm × 52 cm HEPA filter (99.99% ASHRAE efficiency, class H14) (Bernard Dumas S.A.S., Creysse, France) was used in a closed-loop chamber while a 36 cm × 120 cm filter was used in an air purifier. The filters were pretreated to remove water-resistance by washing with 0.08 M sodium dodecyl sulfate (SDS) before dipping into a 2% Degussa P25 TiO₂ suspension and dried at room temperature for 24 hours before calcination at 120°C for 1 hour. TiO₂ loading was determined from the weight difference before and after the coating procedure. Two loadings were investigated, 1,870±169 and 3,140±67 mg/m². These filters were pleated and framed in filter holders and sterilized with UV-C radiation before use.

2.2. Airborne microbe preparation

All microorganisms in this study were purchased from the Microbiological Resource Centre (MIRCEN), Thailand Institute of Scientific and Technological Research, Bangkok, in dried-freeze form. S. epidermidis (TISTR 518) was inoculated at 35-37°C on tryptic soy agar (TSA) plates for 24 hours, while B. subtilis (TISTR 008) was inoculated for sporulation at 37°C for 7 days. The spores were harvested into sterile distilled water, and heated at 80°C for 10 minutes to inactivate vegetative cell and centrifuged at 2,500 rpm for 5 min. P. citrinum (TISTR 3437) was incubated at 25°C for 7 days and the spores were harvested into sterile distilled water and centrifuged at 2,000 rpm for 5 min. A. niger (TISTR 3012) was inoculated on Sabouraud Dextrose Agar (SDA) and grown at 37°C for 3 days. All culture suspensions were diluted in sterile water to the required microorganism concentration of 10⁴-10⁷ CFU/ml (Lin and Li, 2003; Vorha et al., 2006).

2.3. Photocatalytic HEPA filters efficacy investigation

In the first phase, the experiment was performed in a closed-loop chamber (Fig. 1). Two photocatalytic HEPA filters were inserted into the chamber side by side. One was irradiated with UVA, while the other was not. In separate runs, the above mentioned microorganisms were filled in a nebulizer and compressed air, at a pressure of 25-30 psi was applied to spray out the microbes for 20 minutes. The blower drew the air to circulate in the chamber at a velocity of 0.3 m/s, measured with a hot wire anemometer (Airflow Developments Ltd., model TA5-Flexible probe, Minnesota, USA). Relative humidity and temperature were controlled at 45±5% and 25°C, respectively. After 20 minutes of injection, the photocatalytic HEPA filters were pulled out to extract the initial microorganisms remaining in them. New sets of the photocatalytic HEPA filters were inserted and the microorganism injection was restarted for another 20 minutes. At this time, five 36-W UV-A lamps (Sylvania F36W-T8/BLB) were turned on with irradiance...
intensity of 4.85±0.09 mW/cm² for 60, 180, 240, 300, 360, 480 or 600 minutes for A. niger, P. citrinum, and B. subtilis disinfection, and 30, 60, 90, 120, 180 or 240 minutes for S. epidermidis disinfection. After each of the irradiation times of interest, HEPA filters were pulled out to extract microorganisms remaining in the filters. Disinfection efficiency was obtained from comparing the remaining concentration with the initial concentration.

For phase 2, in a chamber (2 m × 2 m × 2 m), a photocatalytic HEPA filter with the same TiO₂ loading was applied to a commercial air purifier (Alpine Co., model PT600, Bangkok, Thailand). The irradiation intensity was 3.18±0.75 mW/cm², measured with a radiometer (Cole Palmer, model LW09811-50, Illinois, USA). The PCO air purifier was located at a corner of the chamber and each type of microorganisms was injected in separate runs using a nebulizer for 6 hours. Microorganisms in the air were collected at the height of 0.5 m and 1.5 m using an Anderson Impactor (SKC Inc., model Standard Biostage, California, USA) with the flow rate of 28.3 L/min and sampling time of 3 minutes every 30 minutes. In the first two hours of injection, the air purifier was still off and was turned on after two hours for 2 hours and turned off again during the last two hours to observe any changes in microorganism concentrations. All sampled plates were incubated immediately at 37°C, 24-48 hours for S. epidermidis, and B. subtilis, 48-72 hours for A. niger, and 25°C, 48 hours for P. citrinum. Relative humidity and temperature were controlled at 45±5% and 25°C, respectively. After each experiment, the chamber was sterilized by UV-C radiation (Sylvania Germicidal lamp 20W, Bangkok, Thailand) with the irradiation intensity of 1.21 mW/cm² for several hours. Inside-surface swabs as well as the bioaerosol sampling were performed to confirm the sterilization.

Phase 3 was conducted in a renal unit of a 800-bed public hospital. A PCO air purifier was located in a renal room (7.2 m W × 12 m L × 3 m H) and a washing room (2 m W × 2 m L × 3 m H). Bacteria and fungi were collected by Anderson Impactor in the same manners as those in phase 2. Three sampling points with a distance from an air purifier of 2 m, 6.5 m, and 8 m were assigned for the renal room and two sampling points with a distance of 1 m and 2 m were assigned for a washing room. The impactor media used for bacteria growth was TSA, while that for fungi was SDA. The media was incubated at 37°C for 24-48 hours. After that, microbial colonies were counted, and calculated in units of CFU/m³.

Descriptive and inferential statistics were performed with SPSS software version 9. A comparison of disinfection efficiency was analyzed using t-test and the analysis of variance (ANOVA) at the confident interval level of 95%.

Figure 2. Morphology of fibers under various conditions (5,000× magnifications for all micrographs)
3. Results and Discussion

The morphology of original HEPA fibers before coating and after coating with two TiO$_2$ loadings, observed by scanning electron microscopy are shown in Fig. 2. We found that TiO$_2$ loading of $3,140 \pm 67$ mg/m$^2$ was immobilized on the fiber better than that of $1,870 \pm 169$ mg/m$^2$ with no little change in pore structure from the original uncoated filter.

Disinfection efficiencies of the photocatalytic HEPA filters on airborne microorganisms in a closed-loop chamber are shown in Fig. 3. We found that the PCO reaction for *S. epidermidis* destruction under low TiO$_2$ loading and high TiO$_2$ loading coated filter could completely destroyed *S. epidermidis* within 4 hours and 2 hours, respectively. There was no significant difference between these two TiO$_2$ loadings ($p=0.239$), but a significant difference occurred between uncoated filter and photocatalytic HEPA filters when all were irradiated ($p=0.0001-0.002$). This indicates that the PCO was more successful for disinfecting *S. epidermidis* than UVA photolysis alone. For *B. subtilis* destruction, PCO reactions from high and low TiO$_2$ loadings were not significantly different ($p=0.063$) Pal et al., 2007 found that increasing TiO$_2$ loading from more than 3,000 mg/m$^2$ to around 9,000 mg/m$^2$ could not increase *B. subtilis* destruction. Increasing TiO$_2$ loading would cause an agglomeration of nano-titania on the surface that could reduce the removal efficiency from the formation of less reactive hydroperoxyl radicals (HO$_2^·$) (Rincon and Pulgarin, 2003). A similar pattern to *B. subtilis* disinfection was found in fungi where 77.1% of *A. niger* and 82.4% of *P. citrinum* were removed under high TiO$_2$ loading at an irradiation time of 10 hours and no significant difference between low and high TiO$_2$ loadings was found.

For the UVA photolysis reaction (uncoated filter with UVA irradiation), UVA was effective only on bacteria but not on fungi, since no significant difference was found between the light and dark section ($p=0.277$ for *A. niger*, $p=0.110$ for *P. citrinum*). This is probably because *S. epidermidis* is a gram-positive bacteria which is sensitive to UV-A light and it has a thick-mesh-like cell wall made of peptidoglycan that is easily destroyed.

Figure 3. Disinfection efficiency of photocatalytic HEPA filters in the closed-loop chamber
Because UV-A has low energy, it could damage the cell by oxidative stress from the oxygen radicals to excite photosensitive molecules within the cell. The excitation produces active species like $\cdot O_2$, $H_2O_2$, and $\cdot OH$ that affect genome and intracellular molecules, leading to inactivated mutations and growth delay (Oguma et al., 2002; Küehn, 2003). It could be implied that fungi in this experiment do not contain photosensitive molecules.

For microorganism removal using a PCO air purifier in the 8-m$^3$ chamber, the results are shown in Fig. 4 under three continuous conditions: (1) background concentrations (air purifier not turned on yet), (2) concentrations during operation of the air purifier and (3) concentrations after turning off the PCO air purifier. When turning on the PCO air purifier at 121 minutes, all microorganisms were decreased immediately and the patterns of reduction efficiencies observed at 0.5 m and 1.5 m were not significantly different ($p=0.954$). On average, 83.4% of $S. \text{epidermidis}$, 81.4% of $B. \text{subtilis}$, 88.5% of $A. \text{niger}$, and 75.8% of $P. \text{citrinum}$ were removed from the air when the air purifier was turned on. On turning off the PCO air purifier, all microorganisms recovered to their initial concentrations. It should be noted that the nebulizer injected the microorganism continuously from 0 minutes to 360 minutes.

For air sampling in the hospital, the mean background concentration of total bacteria in the renal room was 555±83 CFU/m$^3$ while mean total fungi concentration was 21±8 CFU/m$^3$. When the air purifier was turned on (at 90 minutes), total bacteria removed was 63.5%, 23.9%, and 28.2% for a distance of 2 m, 6.5 m, and 8 m from the PCO air purifier, respectively, indicating that the efficacy of the PCO air purifier decreased with increasing distance. When the air purifier was turned off, total bacteria concentration increased immediately for the furthest point (8 m from the air purifier) [Fig. 5(a)]. At that time the door was open for transferring patients and this might cause the ingress of total bacteria to that point which was located in front of the door. For the washing room [Fig. 5(b)], the pattern of reduction efficiency was similar to that of the 8-m$^3$ chamber. The mean background of total bacteria concentrations were 1,125±135 and 1,143±109 CFU/m$^3$ at a distance of 1 m and 2 m from the air purifier, respectively. The levels were higher than those in renal room by approximately 2-3 times due to garbage, hazard wastes and toilets within the washing room. In terms of efficacy of the PCO air purifier, it clearly removed 77.5% and 59.5% of bacteria at a distance of 1 m and 2 m, respectively. The PCO air purifier in this room was more effective than in the renal room.
because the volume of the washing room was smaller than that of renal room by about 21.6 times. When the air purifier was turned off, the bacteria recovered to near the original concentrations [Fig. 5(b)]; therefore, using a PCO air purifier could dramatically reduce the airborne microorganisms, if the distance or the size of the room is appropriate.

When comparing microorganism reduction efficiency of PCO air purifiers under a real situation and in the experimental chamber, the efficacy of in the real situation was lower. This could be the result of higher relative humidity (73-75%) RH in washing room and 74-76% RH in renal room. The relative humidity is the main factor affecting the PCO reaction because at higher humidity water molecules cover the surface of the photocatalytic HEPA filters and occupy the active sites of radical production (Zhao and Yang, 2003); consequently, the efficacy of the PCO reaction is reduced. Normally, the efficiency of PCO is optimal at 50% RH (between 40-70% RH) (Goswami, 2003). Therefore, besides the room size and distance from the air purifier, relative humidity is another factor for consideration.

4. Conclusions

A TiO₂ loading of 1,870±169 mg/m² was not significantly different from the loading of 3,140±67 mg/m² for microorganism disinfection in this study. Photolysis could destroy bacteria, especially *S. epidermidis*, but could not affect fungi, and lacks of photosensitive molecules in their cells were suspected to cause the difference. Disinfection efficiencies of all microorganisms in a closed-loop chamber were in the range of 77.1-100% after 10 hours of irradiation, while those in the 8-m³ chamber were in a range of 72.4-88.5%. However, when applying it to a real unit in a hospital environment, the efficacy was reduced. We suggest that room size, distance from the air purifier, and humidity were the main reasons.

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