Photochemical & Photobiological Sciences

Accepted Manuscript

This is an *Accepted Manuscript*, which has been through the Royal Society of Chemistry peer review process and has been accepted for publication.

Accepted Manuscripts are published online shortly after acceptance, before technical editing, formatting and proof reading. Using this free service, authors can make their results available to the community, in citable form, before we publish the edited article. We will replace this *Accepted Manuscript* with the edited and formatted *Advance Article* as soon as it is available.

You can find more information about *Accepted Manuscripts* in the Information for Authors.

Please note that technical editing may introduce minor changes to the text and/or graphics, which may alter content. The journal's standard Terms & Conditions and the Ethical guidelines still apply. In no event shall the Royal Society of Chemistry be held responsible for any errors or omissions in this *Accepted Manuscript* or any consequences arising from the use of any information it contains.

ROYAL SOCIETY OF CHEMISTRY

www.rsc.org/pps

Photochemical & Photobiological Sciences

Photochemical & Photobiological Sciences

Dynamic Article Links ►

Cite this: DOI: 10.1039/c0xx00000x

ARTICLE TYPE

www.rsc.org/xxxxxx

For inactivation of mold spores by UVC radiation, TiO_2 nanoparticles may act as a "sun block" better than as a photocatalytic disinfectant, and ozone acts as a promoter

Jia-You Gong, Yen-Chi Chen, Yi-Ting Huang, Ming-Chien Tsai and Kuo-Pin Yu*

Received (in XXX, XXX) Xth XXXXXXXXX 20XX, Accepted Xth XXXXXXXXX 20XX
DOI: 10.1039/b000000x

Fungal spores have been known as a critical indoor allergen, and the indoor air purification techniques including titanium dioxide (TiO_2) photocatalytic disinfection, ultraviolet germicidal irradiation (UVGI) and ozonation have been considerably investigated. However, most of the researches regarding photocatalytic disinfection focused on anti-bacterial efficacy of TiO_2 nanoparticles (NPs). Furthermore, some researches even showed that the photocatalytic antifungal efficacy of TiO_2 NPs may be not that significant. Thus, investigating reasons behind the non-significant antifungal efficacy of TiO_2 photocatalytic disinfection and enhancing antifungal efficacy are also indispensable. In this study, ozone was employed to improve the photocatalytic antifungal efficacy of the TiO_2NPs and nano-metal supported by TiO_2NPs. The commercial TiO_2NPs (Defussa (Evonik) P25) served as a good support, and the incipient wetness impregnation was successfully exploited to prepare oxidized nano-metals (Ag, Cu and Ni) in this study. There were two surfaces (quartz and putty) in the inactivation experiments of *Aspergillus niger* spores which were manipulated under two conditions: exposed to ultraviolet light (UVC); exposed to UVC and ozone simultaneously. The SEM images demonstrated that spores were sheltered from the UVC light in the micro crave between TiO_2 agglomerates. When irradiating with UVC, *A. niger* spores on the two testing surfaces without TiO_2NPs was inactivated faster than those with TiO_2NPs, implying a "sun block" effect of this material and a lower photocatalytic antifungal efficacy than UVGI. On both surfaces, the inactivation rate constants (k) of *A. niger* spores exposed to UVC and ozone simultaneously (on quartz: k = 2.09–6.94 h^{-1}, on putty: k = 3.17–6.66 h^{-1}) were better than those exposed to only UVC (on quartz: k = 1.80–5.89 h^{-1}; on putty: k = 2.97–3.98 h^{-1}), indicating ozone can enhance the UVGI antifungal efficacy.

Introduction

Mold spores can cause numerous adverse health effects including irritation, allergy, infection and respiratory diseases[1-3]. The moderation of indoor fungal quantity is crucial for people in the modern society spend 80-90% time of their daily life indoors where the fungal concentration is higher than outdoor [4, 5].

In order to remediate microbial contaminations, the germicidal efficacy of some disinfection methods such as TiO_2-mediated photocatalytic disinfection, nano-metals and ozonation have been extensively investigated[6-25]. However, most of the studies which aim to photocatalytic removal of microbial contamination focused on antibacterial efficacy of photocatalytic disinfection, especially *Escherichia coli*, which is uncommon in indoor air[7, 12, 14-17, 21, 22]. In contrast to bacteria, the cell wall structures of fungal are more complex and make them tough and highly resistance to sterilizers[18]. Some studies on antifungal activities of photocatalytic disinfection mentioned the ultraviolet (UVA) light and TiO_2 phtocatalyst is a potent fungicide combination and the sterilizing effect would vanish when the UVA was removed [6, 24]. The result indicated that the antifungal ability may originate mostly from the UVA light, not the photocatalyst. Many studies have showed that there is only little difference between the antifungal effectiveness of UVC-illuminated surface with and without TiO_2 [20, 25]. Paschoalino et al[23] even found that fungi survived better in UVC-illuminated-TiO_2 photocatalysis (3.5 CFU/plate) than in UVC photolysis (2.0 CFU/plate). Since lots of the photo energy that can be used to kill fungi directly lose during the photochemical conversion process, it is very possible that the antifungal efficacy of photocatalytic disinfection is not as powerful as ultraviolet germicidal irradiation (UVGI).

Ozone is an effective germicide that has been widely used for sterilizing drinking water[26-28]. Being a powerful oxidizer that can inactivate germs, spores and viruses by oxidizing their cell walls and change the cell permeability which leads to cell death, ozone has also been applied in indoor air purification[29, 30]. In addition,

ozone has a higher electron affinity (2.1 eV) as compared with that of O_2 (0.44 eV). Thus, it can capture the photocatalytic electron more efficiently than O_2 and produce additional hydroxyl radicals, $\bullet O_3^-$ and $\bullet O^-$ radicals to inactivate microbes[31]. Besides, ozone can be photolyzed by the UVC radiation and can generate atomic oxygen, $O(^1D)$ ($O_3 \xrightarrow{UV} O_2 + O(^1D)$), and then the $O(^1D)$ will react with water molecules to generate hydroxyl radicals ($O(^1D) + H_2O \rightarrow 2OH\bullet$). In these reactions, ozone is consumed and other reactive oxygen species (ROSs) generate.

This study aims to verify the antifungal effect of TiO_2 nanoparticles (NPs) as a photocatalytic disinfectant and the influence on the antifungal ability of UVGI. *Aspergillus niger* spore was selected as the target spore because it is omnipresent in the indoor environments. We also investigated the enhancement effect of ozone on the UVC antifungal efficacy.

Methodology

Preparing and characterizing nano-metals supported by TiO_2NPs (nano-metals/TiO_2NPs)

The incipient wetness impregnation (IWI) was exploited to prepare oxidized nano-metals supported by TiO_2NPs (nano-metals/TiO_2NPs) in this study. Degussa (Evonik) P25 TiO_2NPs, which is a mixed phase of 30% rutile and 70% anatase with a prime particle size of 30 nm, was used as the support, while silver nitrate ($AgNO_3$ purity 99.99%, J.T Baker, USA), copper (II) nitrate 2.5 hydrate ($Cu(NO_3)_2 \cdot 2.5H_2O$, purity: 99.9%, J.T. Baker, USA), and nickel (II) nitrate hexahydrate ($Ni(NO_3)_2 \cdot 2.5H_2O$, purity: 99.9%, Lot No.: 10156297, Alfa Aesar, UK) were used as the nano-Ag, Cu, Ni precursors in IWI.

In the initial stage, in order to provide appropriate amount of metal, the metal precursor was dissolved in deionized distilled water and the volume was the same volume as the pore volume of TiO_2 powder (0.4 mL of water/per gram of P25 TiO_2). Afterward, the metal-containing solution was dropped slowly into the TiO_2 support, and then the mixture was stirred for 2 hours to be well mixed. In the end, we dried the mixture at 120°C overnight and ground into fine powder with an agate mortar. Each prepared nano-metals/TiO_2NPs is denominated as **X**wt%**Y**/P25, in which **X** is the weight percentage of nano-metals; **Y** is the species of metals. A JSM-7600F scanning electron microscope (SEM) was utilized to characterize the appearance of the TiO_2 support agglomerate. UV-Visible absorbance spectra of the prepared samples were obtained on an UV-Visible/NIR spectrophotometer (Hitachi U-3310, Japan).

Culturing of *A. niger* to yield spores

In the study, the strain of *A. niger* from Bioresources Collection and Research Center (BCRC) in Taiwan (BCRC 30310) was shipped in freeze-dried form and it required activation following the description in the BCRC instruction and plated on male extract agar (MEA, DifcoTM) twice to ensure the purity of the strain before use. After that, the *A. niger* culture was apply to the MEA plate and incubated for more than one week for sporulation. The spores were harvested with autoclaved 0.05% TWEEN® 80 (Sigma-Aldrich Co.) solution on a shaker with the shaking rate of around 80 rpm for ten minutes and then the suspension was collected. The average spores concentration of the suspension was about 10^7 CFU/ml. (CFU= colony forming unit)

Inactivating *A. niger* spores by P25 TiO_2NPs and nano-metals/TiO_2NPs

The inactivation of spores was manipulated under three conditions: (1) exposure to UVC (254 nm, 750 μW/cm^2); (2) exposure to UVC as well as 5 ppm of ozone (UVC+O3) (3) without UVC and ozone exposure (Control), and two surfaces: (1) quartz chip; (2) putty (a kind of building materials consisting of primarily calcium carbonate, some resins and preservatives). In order to provide the UVC source, a UVC lamp was assembled inside the chamber beforehand. Ozone was provided by an ozone generator and it took about 5 minutes for the experimental system to achieve the required level of 5-ppm ozone.

Before the inactivation experiment, the supporting surface (quartz and putty) of P25 TiO_2NPs and nano-metals/TiO_2NPs needed to be prepared. First we added 10 μL of the aqueous suspension which was comprised of 5% P25 TiO_2NPs or nano-metals/TiO_2NPs and 0.005% dioctylsulfossuccinate sodium (DSS, purity: 99.9%, Sigma) on each 0.5 cm × 0.5 cm quartz chip with putty or without putty. Then these chips were baked on a hot plate to produce a uniform coating of P25 TiO_2NPs and nano-metals/TiO_2NPs on the chips. The last step was to sterilize these chips in an autoclave. After preparing chips, 10 μL of suspension of *A. niger* spores (around 10^5 spores) was inoculated on each disinfected chip and then placed into a sterilized stainless steel test chamber which was connected to a zero air system as air supplier. The air supplier provided the chamber air at the circumstance of air change rate of 5 h^{-1} and 50% relative humidity. The experiments did not start until the chips with *A. niger* spores suspension were dry (It took about 1 hr). To harvest the spores at the time of 0, 1, 2, 3 and 4 hour in the condition of exposure to UVC and the condition of exposure to UVC as well as ozone, the chips with spores were put into a spiral tube containing 10 mL of autoclaved 0.05% TWEEN 80 solution and then oscillated with a vortex mixer for about 1 minute. The suspensions were serially diluted, and then cultured on MEA medium at 25°C for 48 hrs. In the end, the CFU on the plates was counted in order to evaluate the viable spores at each time.

We used the D-value to evaluate antifungal efficacy of the P25 TiO_2NPs and nano-metals/TiO_2NPs exposure to UVC as well as ozone. The D-value refers to decimal reduction time and is the time required at a certain condition to kill 90% of *A. niger* spores. In most disinfection studies, the logarithm of the survival ratio (*SR*) of organism is nearly linear proportional to the dose, in which dose is the product of concentration and exposure time for chemical (O_3 and nano-metal) or intensity and exposure time for UVC. Thus, the antifungal kinetics was obtained from the *SR* of culturable *A. niger* spores at different time points. This is expressed mathematically as:

$$SR = \frac{N(t)}{N(0)} = \exp(-kt) \quad (1)$$

in which $N(t)$ and $N(0)$ are the concentrations of culturable *A. niger* spores harvested from chip at time t and at the beginning of the experiment, respectively; k is the inactivation rate constant, which is a function of UVC intensity (in the condition of UVC), ozone concentration, nano-metal loading level (in the condition

of Control) and all above (in the condition of UVC+O3). The total inactivation rates of *A. niger* spores were described in terms of log reduction after 4-hour exposure:

$$\log reduction = \log \frac{N(0)}{N(4hour)} \quad (2)$$

The recovery efficiency (initial survival fraction (*SFi*)) of *A. niger* spores after applying on the surface was calculated as what follows:

$$SFi = \frac{N(0)}{N_{inoculated}} \quad (3)$$

where, $N_{inoculated}$ is the concentration of viable *A. niger* spores inoculated on the quartz chips. The *SFi* on different surface ranged from 0.099±0.019~0.117±0.01.

Results and discussion

Inactivation of the mold spores by P25 TiO_2NPs and nano-metals/TiO_2NPs under UVC irradiation

As shown in Figure 1 (a), no matter which metals/TiO_2NPs were applied to the quartz chip, when exposed to UVC their antifungal efficacy were better than those without UVC exposure. The antifungal efficacy resulted from either UVGI or photocatalytic disinfection, or both of them. In UVGI, the UVC (254 nm) is effective in damaging the nucleic acids of these spores so that their DNA is disturbed, leaving them unable to carry out vital functions. In photocatalytic disinfection, the UV radiation with energy above the band-gap of TiO_2 (wavelength < 385 nm) induces the formation of electron-hole pairs and generates hydroxyl radical further on the UV-illuminated TiO_2 surface, and then the microbial inactivation is mediated by hydroxyl radicals that attacks microbes including bacteria, virus, yeast, even the cysts of protozoa[12-22, 25, 32].

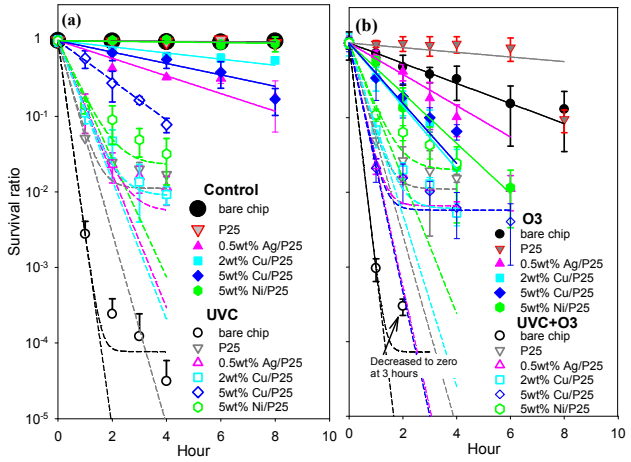


Figure 1. The time profiles of the survival ratio of *A. niger* spores on the surfaces of P25 TiO_2NPs (P25), nano-metals/TiO_2NPs and quartz chip (bare chip) **(a)** without UVC (Control) and under the UVC irradiation (UVC); **(b)** exposed to 5-ppm ozone (O3) and exposed to UVC irradiation and 5-ppm ozone simultaneously (UVC+ O3)

One interesting result found in our experiments was that *A. niger* spores on the quartz chips coated with P25 TiO_2NPs and nano-metals/TiO_2NPs showed a lower inactivation rate (a lower *k* and log reduction in Table 1 and Table 3, respectively, and a larger D-value in Table 2) than those on bare quartz chips. Since the inactivation rate of *A. niger* spores on bare quartz chips obtained in this study was consistent to the result of the previous study[33], indicating our experimental result could be correct. Thus, we suspected that the TiO_2 particles might act as the "sun block" that absorbed and scattered the UVC irradiation and protected *A. niger* spores from the damage caused by UVC, as shown in Figures 2(a) and 2(c).

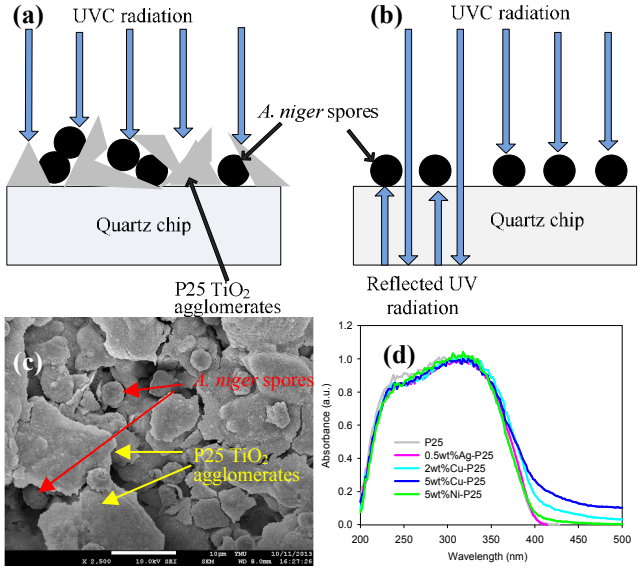


Figure 2. (a) TiO_2 agglomerates can protect *A. niger* spores from the UVC irradiation and prevent the penetration of UVC irradiation and blocked the UVC irradiation reflected from the bottom of quartz chip. (b) Without TiO_2 coating layer, the UVC irradiation can penetrate and then be reflected from the bottom of quartz chip and cause serious damages to *A. niger* spores; (c) SEM image demonstrates that the *A. niger* spores are surrounded by TiO_2 agglomerates; (d) The UV-visible absorbance of P25 TiO_2NPs and nano-metals/TiO_2NPs

As demonstrated in Figure 2(d), P25 TiO_2NPs can absorb UVC efficiently and the UV absorbance of the P25 TiO_2NPs and nano-metals/TiO_2NPs is higher than 0.8, indicating less than 20% of the UVC radiation can be employed to inactivate *A. niger* spores directly. Notably, there were numerous micro-cracks on the coating of TiO_2, and these crevices acted as the shelter and provided asylum for spores. Moreover, in our observation with SEM, some of the *A. niger* spores even sank into those deep and narrow chinks that barely exposed to UVC. Spores stayed in the shade of TiO_2 could escape from the destruction of ultraviolet. Furthermore, the tailing-off inactivation curves of *A. niger* spores in Figure 1 could also be explained by the survival of a resistant subpopulation because of the protection by interfering substances (P25 TiO_2NPs and nano-metals/TiO_2NPs). In addition, the complex envelop of *A. niger* spores may mitigate the antifungal efficacy of TiO_2-mediated photocatalytic disinfection, leading to the lower efficacy than UVGI. Consequently, the inactivation rate of *A. niger* spores on the bare quartz chips was higher than that on the surface of P25 TiO_2NPs and nano-metals/TiO_2NPs. A similar result was also reported by Paschoalino et al.[23] using a

polyester supported TiO_2 photo-reactor for indoor air disinfection. They found that the survival of fungi in UVC-illuminated-TiO_2 photocatalysis (3.5 CFU/plate) was better than in UVC photolysis (2.0 CFU/plate). Chen et al.[25] discovered that TiO_2 has only little effect on the inactivation of fungal spores on the wet wooden surface. Thus, in our experiments, the inactivation effectiveness on *A. niger* spores was primarily provoked by the strong germicidal effect of UVC and partially caused by photocatalytic disinfection. Additionally, loading nano-metals on TiO_2 would lead to the decrease of inactivation rate as shown in Figure 1(a) and Tables 1-3, and the more loading level of nano-metals, the lower the inactivation rate (inactivation rate of 5wt% Cu-P25 < 2wt% Cu-P25). Paschoalino et al.[23] also reported a similar result that fungi survived better in UVC-illuminated-TiO_2/Ag photocatalysis (3.7 CFU/plate) than in UVC photolysis (1.3 CFU/plate) and a little better than in UVC-illuminated-TiO_2 photocatalysis (3.5 CFU/plate compared to UVC photolysis: 2.0 CFU/plate). This may be owing to that the nano-metals can improve the recombination of electron-hole pairs generated from the photocatalytic reaction and reduce the production of hydroxyl radicals that attack the microbes[34, 35].

We also tested the antifungal effectiveness of P25 TiO_2NPs and nano-metals/TiO_2NPs on the putty surface. As shown in Figure 3 (a) and Tables 1-3, when exposed to UVC radiation, the inactivation rates of *A. niger* spores on the putty surface was lower than those on the quartz chips, signifying that the putty may also has the "sun block" effect on the UVC irradiation. And this "sun block" effect was enhanced when putty and P25 TiO_2NPs was applied simultaneously. On the other hand, owing to the antifungal effect of nano-metals, the inactivation rates of *A. niger* spores on the surface of nano-metals/TiO_2NPs were higher than those on the surface of TiO_2NPs.

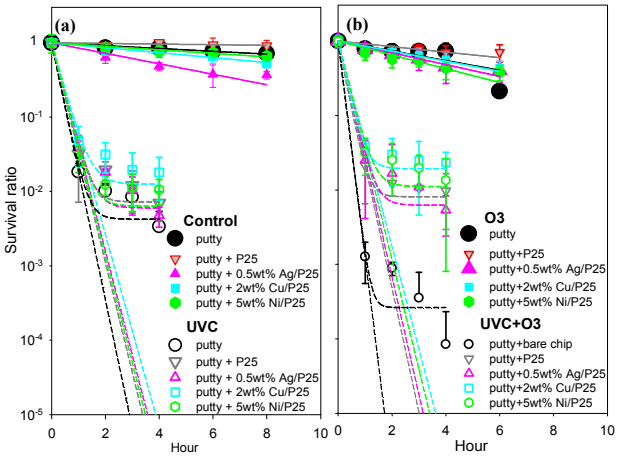


Figure 3. The antifungal kinetics of P25 TiO_2NPs (P25) and nano-metals/TiO_2NPs against *A. niger* spores when applied on the putty **(a)** without UVC (Control) and under the UVC irradiation (UVC); **(b)** exposed to 5-ppm ozone (O3) and exposed to UVC irradiation and 5-ppm ozone simultaneously (UVC+ O3)

Inactivation of mold spores by P25 TiO_2NPs and nano-metals/TiO_2NPs exposed to UVC and ozone simultaneously

In order to verify whether ozone has a promotional effect on photocatalytic disinfection and UVGI, these three techniques (O_3; photocatalytic disinfection; UVGI) were exploited together in this study. In our experimental system, the ozone concentration decreased from 5 to 3.5 ppm during the reactions and transformed to other ROSs (including •OH, $•O_3^-$ and $•O^-$ radicals) and all of these ROSs could cause damages to *A. niger* spores and therefore led to inactivation.

Table 1. The inactivation rate constant, k, (unit: h^{-1}) of *A. niger* spores under various conditions

Condition	Control**	UVC	O3**	UVC+O3
Bare chip	-	5.89	0.304	6.94
P25 TiO_2	0.005	2.92	0.072	2.95
0.5wt% Ag-P25	0.268	2.03	0.475	3.75
2wt% Cu-P25	0.093	2.12	0.966	2.65
5wt% Cu-P25	0.173	0.605	0.929	3.83
5wt% Ni-P25	0.01	1.80	0.77	2.09
Putty	0.044	3.98	0.150	6.66
P25 TiO_2 on putty	0.009	3.40	0.085	3.82
0.5wt% Ag-P25 on putty	0.162	3.22	0.182	3.64
2wt% Cu-P25 on putty	0.076	2.97	0.156	3.17
5wt% Ni-P25 on putty	0.051	3.3	0.214	3.38

**The data of these two conditions were adopted from our previous publication for comparison[6]

Table 2. The D-value (hour) of *A. niger* spores at various conditions

Condition	Control	UVC	O3	UVC+O3
Bare chip		0.39	7.57#	0.33
P25 TiO_2	>100#	0.79	8.00	0.78
0.5wt% Ag-P25	8.59#	1.13	4.85	0.61
2wt% Cu-P25	24.8#	1.09	2.38	0.87
5wt% Cu-P25	13.3#	3.81	2.48	0.60
5wt% Ni-P25	>100#	1.28	2.99	1.10
Putty	>30#	0.58	15.4#	0.35
P25 TiO_2 on putty	>100#	0.68	7.50	0.60
0.5wt% Ag-P25 on putty	14.2#	0.72	12.7#	0.63
2wt% Cu-P25 on putty	>30#	0.78	14.8#	0.73
5wt% Ni-P25 on putty	>30#	0.70	10.8#	0.68

The D-values larger than the time of experiment were estimated by the inactivation rate constant (D-value = ln10/k)

Table 3. The log reduction of *A. niger* spores after 4-hour exposure

Condition	Control	UVC	O3	UVC+O3
Bare chip	0	4.5	0.5	∞
P25 TiO_2	0	1.8	0	1.8
0.5wt% Ag-P25	0.5	1.9	1.0	2.2
2wt% Cu-P25	0.2	2.0	1.7	2.3
5wt% Cu-P25	0.2	1.1	1.2	2.2
5wt% Ni-P25	0	1.5	1.4	1.6
Putty	0.1	2.5	0.1	4.1
P25 TiO_2 on putty	0	2.2	0.2	2.0
0.5wt% Ag-P25 on putty	0.4	2.3	0.3	2.3
2wt% Cu-P25 on putty	0.2	1.7	0.3	1.6
5wt% Ni-P25 on putty	0.1	2.0	0.4	1.9

As shown in Tables 1-3, the inactivation rates of *A. niger* spores exposed to the UVC irradiation and ozone simultaneously (UVC+O3) were larger than those only exposed to the ultraviolet irradiation (UVC) or only exposed to ozone (O3). Thus, ozone has a promotional effect on the inactivation of *A. niger* spores by photocatalytic disinfection and UVGI. Furthermore, the nano-metal oxide could considerably improve the antifungal efficacy of ozone via generating hydroxyl radical (OH·) [6]:

$O_3+◎ \rightarrow ◎O^- +O_2$ (4)

$◎O^-+H_2O \rightarrow 2OH\cdot$ (5)

where ◎ is the active site of nano-metals oxide.

However, similar to the results observed in the condition of UVC, the inactivation rate of *A. niger* spores in the condition of UVC+O3 would become lower in the presence of P25 TiO_2NPs or nano-metals/TiO_2NPs. This might be owing to the protection provided by the agglomerates of P25 TiO_2NPs and nano-metals/TiO_2NPs for *A. niger* spores against the UVC irradiation. Again, the obvious tailing-off inactivation curves implied the survival of a resistant subpopulation of *A. niger* spores owing to the protection by interfering substances (P25 TiO_2 and nano-metals/TiO_2NPs), clumping, or generally conferred resistant[36]. Owing to the tailing-off curve, some of the inactivation rates evaluated by log reduction (Table 3) were not consistent with those estimated by inactivation rate constant and D-value (Tables 1 and 2).

The antifungal experiments were also conducted on the putty surface. Owing to the "sun block" effect of putty against the UVC radiation, the inactivation rates of *A. niger* spores on the putty surface was lower than those on the quartz chips when exposed to UVC and ozone the simultaneously, as shown in Figure 3 (b), Tables 1-3. Moreover, when putty and P25 TiO_2NPs were applied simultaneously, this protection effect was enhanced. Additionally, because the nano-metals can enhance the recombination of electron-hole pairs and decrease the production of oxidative hydroxyl radicals for the photocatalytic disinfection [34, 35], the inactivation rates of *A. niger* spores on the surface of nano-metals/TiO_2NPs were lower than that on the surface of P25 TiO_2NPs.

Limitations

TiO_2 concentration (relate to TiO_2 distribution on surface) and UV wavelength (UVA or UVC) and intensity might affect the antifungal effectiveness and might alter the "sun block" effect. However, these two effects were not considered in the present study and could be a work of future study.

Conclusions

TiO_2NPs and nano-metals/TiO_2NPs may function as the "sun block" in UVGI experiment and the photocatalytic antifungal efficacy is not as powerful as UVGI, so that the disinfection effectiveness is abated. Furthermore, the nano-metals can enhance the recombination of photocatalytic generated electron-hole pairs and reduce the production of hydroxyl radicals, leading to the decrease of inactivation rates of *A. niger* spores. On the contrary, ozone can improve the antifungal efficacy of nano-metal, TiO_2 photocatalyst and UVGI via the production of additional reactive radicals.

Acknowledgement

The authors would like to thank the financial support provided by the National Science Council of Taiwan under contract number NSC 99-2211-E-010-005- and a grant from Ministry of Education, Aiming for the Top University Plan. We also would like to appreciate the Institute of Anatomy & Cell Biology of National Yang-Ming University for SEM image recording. We also would like to thank Dr. Shu-Hua Chien and Institute of Chemistry, Academia Sinica for UV-Visible absorbance spectra measurement and Ms. Jiayu Chen for English editing.

Notes and references

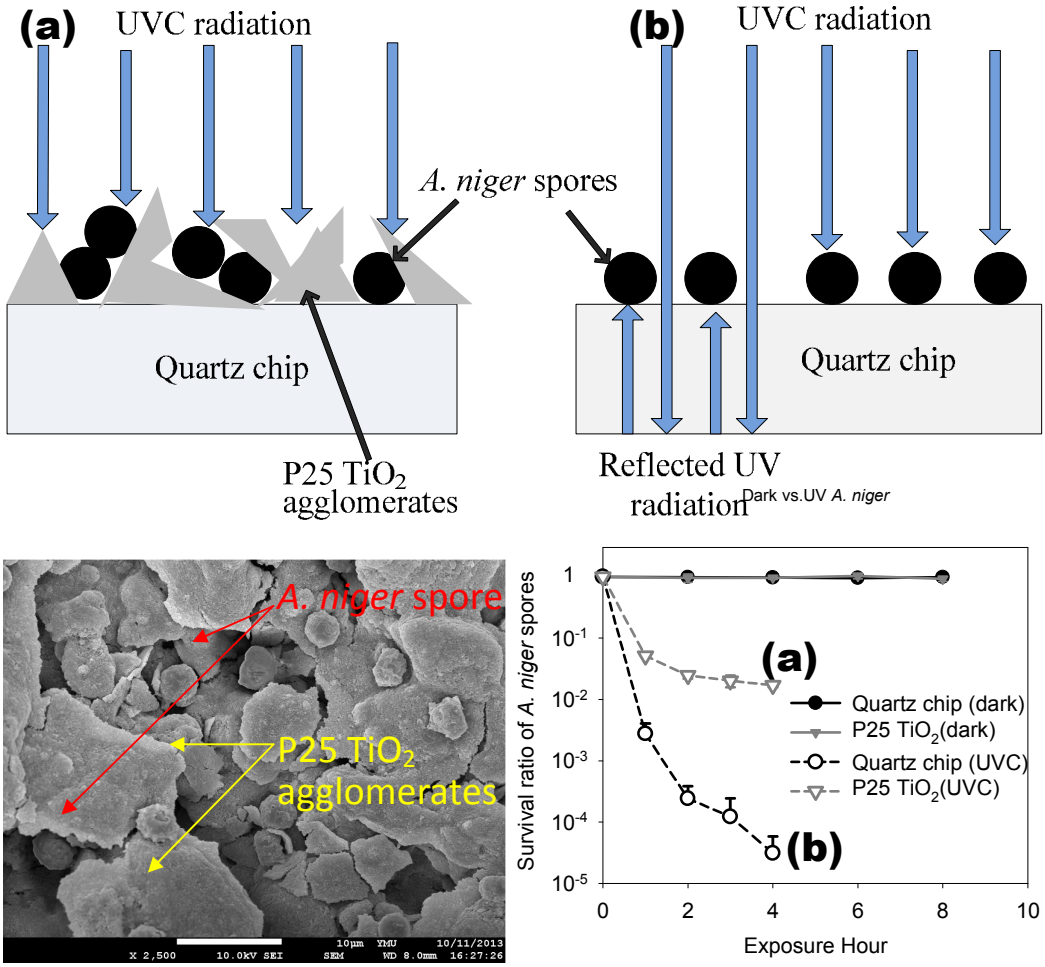
** Corresponding author*
No.155, Sec.2, Linong Street, Taipei, 11221 Taiwan. Fax: +886-2-28278254; Tel:+886-2-28267933; E-mail: kpyu@ym.edu.tw

1. A. Pringle, Asthma and the Diversity of Fungal Spores in Air, *PLoS Pathogens*, 2013, **9**, e1003371.
2. H. A. Burge, An update on pollen and fungal spore aerobiology, *Journal of Allergy and Clinical Immunology*, 2002, **110**, 544-552.
3. F. Fung and W. G. Hughson, Health effects of indoor fungal bioaerosol exposure, *Applied Occupational and Environmental Hygiene*, 2003, **18**, 535-544.
4. J. D. Spengler, J. M. Samet and J. F. McCarthy, *Indoor Air Quality Handbook*, McGraw-Hill, 2001.
5. L. S. Ruzer and N. H. Harley, *Aerosols Handbook: Measurement, Dosimetry, and Health Effects*, CRC press, 2012.
6. K.-P. Yu, Y.-T. Huang and S.-C. Yang, The antifungal efficacy of nano-metals supported TiO_2 and ozone on the resistant *Aspergillus niger* spore, *Journal of Hazardousl Materials*, 2013, **261**, 155-162.
7. A. Rincon and C. Pulgarin, Photocatalytical inactivation of *E. coli*: effect of (continuous-intermittent) light intensity and of (suspended-fixed) TiO_2 concentration, *Applied Catalysis B: Environmental*, 2003, **44**, 263-284.
8. L. Rizzello, R. Cingolani and P. P. Pompa, Nanotechnology tools for antibacterial materials, *Nanomedicine*, 2013, **8**, 807-821.
9. L. Visai, L. De Nardo, C. Punta, L. Melone, A. Cigada, M. Imbriani and C. R. Arciola, Titanium oxide antibacterial surfaces in biomedical devices, *The International Journal of Artificial Organs*, 2011, **34**, 929.
10. Y. Wei, S. Chen, B. Kowalczyk, S. Huda, T. P. Gray and B. A. Grzybowski, Synthesis of stable, low-dispersity copper nanoparticles and nanorods and their antifungal and catalytic properties, *The Journal of Physical Chemistry C*, 2010, **114**, 15612-15616.
11. J. Gamage and Z. Zhang, Applications of Photocatalytic Disinfection, *International Journal of Photoenergy*, 2010, **2010**, 1-11.
12. K.-P. Yu, G. W.-M. Lee, S.-Y. Lin and C. P. Huang, Removal of bioaerosols by the combination of a photocatalytic filter and negative air ions,, *Journal of Aerosol Science*, 2008, **79**, 377-392.
13. T. Matsunaga, R. Tomoda, T. Nakajima and H. Wake, Photoelectrochemical sterilization of microbial cells by

semiconductor powders, *FEMS Microbiology Letters*, 1985, **29**, 211-214.

14. J. C. Ireland, P. Klostermann, E. W. Rice and R. M. Clark, Inactivation of *Escherichia coli* by Titanium Dioxide Photocatalytic Oxidation, *Applied and Environmental Microbiology*, 1993, **59**, 1668-1670.
15. W. A. Jacoby, P. C. Maness, E. J. Wolfrum, D. M. Blake and J. A. Fennell, Mineralization of Bacterial Cell Mass on a Photocatalytic Surface in Air, *Environmental Science & Technology*, 1998, **32**, 2650-2653.
16. K. Sunada, Y. Kikuchi, K. Hashimoto and A. Fujishima, Bactericidal and detoxification effects of TiO_2 thin film photocatalysts, *Environmental Science & Technology*, 1998, **32**, 726-728.
17. P.-C. Maness, S. Smolinski, D. M. Blake, Z. Huang, E. J. Wolfrum and W. A. Jacoby, Bactericidal Activity of Photocatalytic TiO_2 Reaction: toward an Understanding of Its Killing Mechanism, *Applied and Environmental Microbiology*, 1999, **65**, 4094-4098.
18. E. J. Wolfrum, J. Huang, D. M. Blake, P.-C. Maness, Z. Huang, J. Fiest and W. A. Jacoby, Photocatalytic Oxidation of Bacteria, Bacterial and Fungal Spores, and Model Biofilm Components to Carbon Dioxide on Titanium Dioxide-Coated Surfaces, *Environmental Science & Technology*, 2002, **36**, 3412-3419.
19. C.-S. Li, C.-C. Tseng, H.-H. Lai and C.-W. Chang, Ultraviolet Germicidal Irradiation and Titanium Dioxide Photocatalyst for Controlling *Legionella pneumophila*, *Aerosol Science and Technology*, 2003, **37**, 961-966.
20. C.-Y. Lin and C.-S. Li, Inactivation of Microorganisms on the Photocatalytic Surfaces in Air, *Aerosol Science and Technology*, 2003, **37**, 939-946.
21. M. Cho, H. Chung, W. Choi and J. Yoon, Linear correlation between inactivation of E. coli and OH radical concentration in TiO_2 photocatalytic disinfection, *Water Research*, 2004, **38**, 1069-1077.
22. M. Cho, H. Chung, W. Choi and J. Yoon, Different Inactivation Behaviors of MS-2 Phage and *Escherichia coli* in TiO_2 Photocatalytic Disinfection *Applied and Environmental Microbiology*, 2005, **71**, 270-275.
23. M. Paschoalino and W. Jardim, Indoor air disinfection using a polyester supported TiO_2 photo-reactor, *Indoor Air*, 2008, **18**, 473-479.
24. F. Chen, X. Yang and Q. Wu, Antifungal capability of TiO_2 coated film on moist wood, *Building and Environment*, 2009, **44**, 1088-1093.
25. F. Chen, X. Yang and Q. Wu, Photocatalytic oxidation of Escherischia coli, Aspergillus niger, and formaldehyde under different ultraviolet irradiation conditions, *Environmental Science & Technology*, 2009, **43**, 4606-4611.
26. P. Foegeding, Ozone inactivation of *Bacillus* and *Clostridium* spore populations and the importance of the spore coat to resistance, *Food Microbiology*, 1985, **2**, 123-134.
27. L. Restaino, E. W. Frampton, J. B. Hemphill and P. Palnikar, Efficacy of ozonated water against various food-related microorganisms, *Applied and Environmental Microbiology*, 1995, **61**, 3471-3475.
28. V. Camel and A. Bermond, The use of ozone and associated oxidation processes in drinking water treatment, *Water Research*, 1998, **32**, 3208-3222.
29. M. F. Boeniger, Use of ozone generating devices to improve indoor air quality, *American Industrial Hygiene Association*, 1995, **56**, 590-598.
30. W. J. Kowalski, *Aerobiological Engineering Handbook*, McGraw-Hill, 2006.
31. K.-P. Yu and G. W. M. Lee, Decomposition of gas-phase toluene by the combination of ozone and photocatalytic oxidation process (TiO_2/UV, TiO_2/UV/O_3, and UV/O_3), *Applied Catalysis B: Environmental*, 2007, **75**, 29-38.
32. C. McCullagh, J. M. C. Robertson, D. W. Bahnemann and P. K. J. Robertson, The application of TiO_2 photocatalysis for disinfection of water contaminated with pathogenic micro-organisms: a review, *Research on Chemical Intermediates*, 2007, **33**, 359-375.
33. L. W. Durrell and L. M. Shields, Fungi Isolated in Culture from Soils of the Nevada Test Site, *Mycologia*, 1960, **52**, 636-641.
34. M. Anpo and M. Takeuchi, The design and development of highly reactive titanium oxide photocatalysts operating under visible light irradiation, *Journal of Catalysis*, 2003, **216**, 505-516.
35. S. Ikeda, N. Sugiyama, B. Pal, G. Marcí, L. Palmisano, H. Noguchi, K. Uosaki and B. Ohtani, Photocatalytic activity of transition-metal-loaded titanium (IV) oxide powders suspended in aqueous solutions: Correlation with electron–hole recombination kinetics, *Physical Chemistry Chemical Physics*, 2001, **3**, 267-273.
36. R. M. Maier, I. L. Pepper and C. P. Gerba, *Environmental Microbiology*, Academic press, second edition edn., 2009.

Graphical Abstract



(a) TiO_2 agglomerates can protect *A. niger* spores from UVC irradiation and prevent the UVC penetration (b) Without TiO_2 protection, UVC irradiation can cause serious damages to *A. niger* spores