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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*

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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$_2$ NPs and nano-metal supported by TiO$_2$ NPs. The commercial TiO$_2$ NPs (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$_2$ NPs was inactivated faster than those with TiO$_2$NPs, 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$_2$ photocatalyst 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 UV-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 O₂ (0.44 eV). Thus, it can capture the photocatalytic electron more efficiently than O₂ and produce additional hydroxyl radicals, •O₂⁻ and •O radicals to inactivate microbes. Besides, ozone can be photolyzed by the UVC radiation and can generate atomic oxygen, O(1D) (O(1D) → O(0) + O(^3)D), and then the O(1D) will react with water molecules to generate hydroxyl radicals (O(1D)+H₂O → 2OH*). In these reactions, ozone is consumed and other reactive oxygen species (ROSs) generate.

This study aims to verify the antifungal effect of TiO₂ nanoparticles (NPs) as a photocatalytic disinfectant and the influence on the antifungal ability of UGVI. 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 TiO₂NPs (nano-metals/TiO₂NPs)

The incipient wetness impregnation (IWI) was exploited to prepare oxidized nano-metals supported by TiO₂NPs (nano-metals/TiO₂NPs) in this study. Degussa (Evonik) P25 TiO₂NPs, which is a mixed phase of 30% rutile and 70% anatase with a purity: 99.9%, Lot No.: 10156297, Alfa Aesar, UK) were used as the support, while silver nitrate (AgNO₃, purity 99.99%, J.T. Baker, USA), copper (II) nitrate 2.5 hydrate (Cu(NO₃)₂·2.5H₂O, purity: 99.9%, J.T. Baker, USA), and nickel (II) nitrate hexahydrate (Ni(NO₃)₂·6H₂O, 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 as the pore volume of TiO₂ powder (0.4 mL of water/per gram of P25 TiO₂). Afterward, the metal-containing solution was dropped slowly into the TiO₂ 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/TiO₂NPs was denominated as Xwt%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 TiO₂ 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 malt 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 10⁶ CFU/ml. (CFU= colony forming unit)

Inactivating A. niger spores by P25 TiO₂NPs and nano-metals/TiO₂NPs

The inactivation of spores was manipulated under three conditions: (1) exposure to UVC (254 nm, 750 µW/cm²); (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 TiO₂NPs and nano-metals/TiO₂NPs needed to be prepared. First we added 10 µL of the aqueous suspension which was comprised of 5% P25 TiO₂NPs or nano-metals/TiO₂NPs and 0.005% dioctylsulfosuccinate 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 TiO₂NPs and nano-metals/TiO₂NPs 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 10⁶ 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 TiO₂NPs and nano-metals/TiO₂NPs 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 (O₃ and nano-metal) or intensity and exposure time for UVC. Thus, the antifungal kinetics was obtained from the SR of cultivable A. niger spores at different time points. This is expressed mathematically as:

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

in which N(t) and N(0) are the concentrations of cultivable 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:

$$\text{log reduction} = \log \frac{N(0)}{N(N\text{hour})}$$  \hspace{1cm} (2)

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

$$\text{SFi} = \frac{N(0)}{N_{\text{ inoculated}}}$$  \hspace{1cm} (3)

where, $N_{\text{ inoculated}}$ 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$_2$NPs and nano-metals/TiO$_2$NPs under UVC irradiation**

As shown in Figure 1 (a), no matter which metals/TiO$_2$NPs 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.$^{12-22, 25, 32}$

![Time profiles of the survival ratio of A. niger spores](image)

Figure 1. The time profiles of the survival ratio of A. niger spores on the surfaces of P25 TiO$_2$NPs (P25), nano-metals/TiO$_2$NPs 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$_2$NPs and nano-metals/TiO$_2$NPs 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$^{31}$, 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).

![Graphs of A. niger spore survival ratio](image)

**Photocatalytic disinfection**

As demonstrated in Figure 2(d), P25 TiO$_2$NPs can absorb UVC efficiently and the UV absorbance of the P25 TiO$_2$NPs and nano-metals/TiO$_2$NPs 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$_2$NPs and nano-metals/TiO$_2$NPs). 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$_2$NPs and nano-metals/TiO$_2$NPs. A similar result was also reported by Paschoalino et al.$^{31}$ using a

![UV-visible absorbance of P25 TiO$_2$NPs and nano-metals/TiO$_2$NPs](image)

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 UV 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$_2$NPs and nano-metals/TiO$_2$NPs
polyester supported TiO₂ photo-reactor for indoor air disinfection. They found that the survival of fungi in UVC-illuminated-TiO₂ photocatalysis (3.5 CFU/plate) was better than in UVC photolysis (2.0 CFU/plate). Chen et al. discovered that TiO₂ has only a 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 TiO₂ 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. also reported a similar result that fungi survived better in UVC-illuminated-TiO₂/Ag photocatalysis (3.7 CFU/plate) than in UVC photolysis (1.3 CFU/plate) and a little better than in UVC-illuminated-TiO₂ photocatalysis (3.5 CFU/plate compared to UVC photolysis: 2.0 CFU/plate). This may be owing to 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.

We also tested the antifungal effectiveness of P25 TiO₂ NPs and nano-metals/TiO₂ NPs 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 TiO₂ NPs were 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/TiO₂ NPs were higher than those on the surface of TiO₂ NPs.

Figure 3. The antifungal kinetics of P25 TiO₂ NPs (P25) and nano-metals/TiO₂ NPs 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 TiO₂ NPs and nano-metals/TiO₂ NPs exposed to UVC and ozone simultaneously

In order to verify whether ozone has a promotional effect on photocatalytic disinfection and UVGI, these three techniques (O₂; 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, •O₂⁻ and •O²⁻ 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

<table>
<thead>
<tr>
<th>Condition</th>
<th>Control</th>
<th>UVC</th>
<th>O₃</th>
<th>UVC+O₃</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bare chip</td>
<td>-</td>
<td>5.89</td>
<td>0.30</td>
<td>6.94</td>
</tr>
<tr>
<td>P25 TiO₂</td>
<td>0.005</td>
<td>2.92</td>
<td>0.072</td>
<td>2.95</td>
</tr>
<tr>
<td>0.5wt% Ag-P25</td>
<td>0.268</td>
<td>2.03</td>
<td>0.475</td>
<td>3.75</td>
</tr>
<tr>
<td>2wt% Cu-P25</td>
<td>0.093</td>
<td>2.12</td>
<td>0.966</td>
<td>2.65</td>
</tr>
<tr>
<td>5wt% Cu-P25</td>
<td>0.173</td>
<td>0.605</td>
<td>0.929</td>
<td>3.83</td>
</tr>
<tr>
<td>5wt% Ni-P25</td>
<td>0.01</td>
<td>1.80</td>
<td>0.77</td>
<td>2.09</td>
</tr>
<tr>
<td>Putty</td>
<td>0.044</td>
<td>3.98</td>
<td>0.150</td>
<td>6.66</td>
</tr>
<tr>
<td>P25 TiO₂ on putty</td>
<td>0.009</td>
<td>3.40</td>
<td>0.085</td>
<td>3.82</td>
</tr>
<tr>
<td>0.5wt% Ag-P25 on putty</td>
<td>0.162</td>
<td>3.22</td>
<td>0.182</td>
<td>3.64</td>
</tr>
<tr>
<td>2wt% Cu-P25 on putty</td>
<td>0.076</td>
<td>2.97</td>
<td>0.156</td>
<td>3.17</td>
</tr>
<tr>
<td>5wt% Ni-P25 on putty</td>
<td>0.051</td>
<td>3.3</td>
<td>0.214</td>
<td>3.38</td>
</tr>
</tbody>
</table>

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

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

<table>
<thead>
<tr>
<th>Condition</th>
<th>Control</th>
<th>UVC</th>
<th>O₃</th>
<th>UVC+O₃</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bare chip</td>
<td>-0.39</td>
<td>7.57⁶</td>
<td>0.33</td>
<td></td>
</tr>
<tr>
<td>P25 TiO₂</td>
<td>&gt;10⁶</td>
<td>7.79</td>
<td>8.00</td>
<td>0.78</td>
</tr>
<tr>
<td>0.5wt% Ag-P25</td>
<td>8.59⁶</td>
<td>1.13</td>
<td>4.85</td>
<td>0.61</td>
</tr>
<tr>
<td>2wt% Cu-P25</td>
<td>24.8⁶</td>
<td>1.09</td>
<td>2.38</td>
<td>0.87</td>
</tr>
<tr>
<td>5wt% Cu-P25</td>
<td>13.3⁶</td>
<td>3.81</td>
<td>2.48</td>
<td>0.60</td>
</tr>
<tr>
<td>5wt% Ni-P25</td>
<td>&gt;10⁶</td>
<td>1.28</td>
<td>3.99</td>
<td>1.10</td>
</tr>
<tr>
<td>Putty</td>
<td>&gt;30⁶</td>
<td>0.58</td>
<td>15.4⁶</td>
<td>0.35</td>
</tr>
<tr>
<td>P25 TiO₂ on putty</td>
<td>&gt;10⁶</td>
<td>0.68</td>
<td>7.50</td>
<td>0.60</td>
</tr>
<tr>
<td>0.5wt% Ag-P25 on putty</td>
<td>14.2⁶</td>
<td>0.72</td>
<td>12.7³</td>
<td>0.63</td>
</tr>
<tr>
<td>2wt% Cu-P25 on putty</td>
<td>&gt;30⁶</td>
<td>0.78</td>
<td>14.8³</td>
<td>0.73</td>
</tr>
<tr>
<td>5wt% Ni-P25 on putty</td>
<td>&gt;30⁶</td>
<td>0.70</td>
<td>10.8³</td>
<td>0.68</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Condition</th>
<th>Control</th>
<th>UVC</th>
<th>O₃</th>
<th>UVC+O₃</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bare chip</td>
<td>0</td>
<td>4.5</td>
<td>0.5</td>
<td>∞</td>
</tr>
<tr>
<td>P25 TiO₂</td>
<td>0</td>
<td>1.8</td>
<td>0</td>
<td>1.8</td>
</tr>
<tr>
<td>0.5wt% Ag-P25</td>
<td>0.5</td>
<td>1.9</td>
<td>1.0</td>
<td>2.2</td>
</tr>
<tr>
<td>2wt% Cu-P25</td>
<td>0.2</td>
<td>2.0</td>
<td>1.7</td>
<td>2.3</td>
</tr>
<tr>
<td>5wt% Cu-P25</td>
<td>0.2</td>
<td>1.1</td>
<td>1.2</td>
<td>2.2</td>
</tr>
<tr>
<td>5wt% Ni-P25</td>
<td>0</td>
<td>1.5</td>
<td>1.4</td>
<td>1.6</td>
</tr>
<tr>
<td>Putty</td>
<td>0.1</td>
<td>2.5</td>
<td>0.1</td>
<td>4.1</td>
</tr>
<tr>
<td>P25 TiO₂ on putty</td>
<td>0</td>
<td>2.2</td>
<td>0.2</td>
<td>2.0</td>
</tr>
<tr>
<td>0.5wt% Ag-P25 on putty</td>
<td>0.4</td>
<td>2.3</td>
<td>0.3</td>
<td>2.3</td>
</tr>
<tr>
<td>2wt% Cu-P25 on putty</td>
<td>0.2</td>
<td>1.7</td>
<td>0.3</td>
<td>1.6</td>
</tr>
<tr>
<td>5wt% Ni-P25 on putty</td>
<td>0.1</td>
<td>2.0</td>
<td>0.4</td>
<td>1.9</td>
</tr>
</tbody>
</table>
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)·:

\[
10. O_3 + O_3 \rightarrow O_3 + O_3 \quad (4)
\]

\[
11. O_3 + H_2O \rightarrow 2OH- \quad (5)
\]

where \(O_3\) is the active site of nano-metals oxide.

However, similar to the results observed in the condition of UV, the inactivation rate of *A. niger* spores in the condition of UVC+O3 would become lower in the presence of P25 TiO2 NPs or nano-metals/TiO2 NPs. This might be owing to the protection provided by the agglomerates of P25 TiO2 NPs and nano-metals/TiO2 NPs 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 TiO2 and nano-metals/TiO2 NPs), clumping, or generally conferred resistant. 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 TiO2 NPs 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, the inactivation rates of *A. niger* spores on the surface of nano-metals/TiO2 NPs were lower than that on the surface of P25 TiO2 NPs.

**Limitations**

TiO2 concentration (relate to TiO2 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**

TiO2 NPs and nano-metals/TiO2 NPs 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, TiO2 photocatalyst and UVGI via the production of additional reactive radicals.

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**Notes and references**

*Corresponding author
No.155, Sec.2, Linong Street, Taipei, 11221 Taiwan. Fax: +886-2-28276254; Tel:+886-2-28267933; E-mail: kpyu@ym.edu.tw

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Graphical Abstract

(a) TiO₂ agglomerates can protect *A. niger* spores from UVC irradiation and prevent the UVC penetration (b) Without TiO₂ protection, UVC irradiation can cause serious damages to *A. niger* spores