

Catalytic inactivation of SARS coronavirus, *Escherichia coli* and yeast on solid surface

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Abstract

Catalytic oxidation is a potential way to disinfect air through a air-condition system. We find that the SARS coronavirus, bacteria and yeast are completely inactivated in 5 min on Ag catalyst surface and in 20 min on Cu catalyst surface at room temperature in air. Scanning electron microscopy (SEM) images show that the yeast cells are dramatically destructed on the Ag/Al₂O₃ and Cu/Al₂O₃ surfaces, which indicates that the inactivation is caused by catalytic oxidation rather than by toxicity of heavy metals.

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1. Introduction

Since the outbreak of severe acute respiratory syndrome (SARS) in south of China was recognized at the end of February 2003, a large amount of chemical disinfectors have been used in epidemic area, which has caused public concern on human health and environment. And further, the safety of using air-condition in SARS lazarettos has become an urgent problem with the coming of summer. Photo catalysis by titanium dioxide (TiO₂) is an alternative to conventional chemical disinfectors [1,2]. However, this technology can only work with ultraviolet light and requires a relatively complex device. On the other hand, surface science and catalysis studies show that oxygen molecules can adsorb and dissociate into active oxygen atoms on some metal surfaces [3,4], and the oxidation of CO and volatile chemical compounds occurs over some supported metal catalysts at room temperature [5–7]. These facts strongly suggest that those catalysts are very promising to be used in air-condition systems for air disinfection. Here,

we report the inactivation efficiency of Ag/Al₂O₃ and Cu/Al₂O₃ to SARS coronavirus, bacteria and yeast.

2. Experimental

The supported catalysts, Ag/Al₂O₃ (Ag 5 wt%) and Cu/Al₂O₃ (Cu 10 wt%) were prepared by an impregnation method. The wet sample was dried at 393 K for 12 h, and then calcined in air at 873 K for 3 h. Before using, the Ag/Al₂O₃, Cu/Al₂O₃ and Al₂O₃ powders were pressed into wafers of ca. 20 mg/cm².

3. Results and discussion

To address inactivation efficiency of Ag/Al₂O₃ and Cu/Al₂O₃, *Escherichia coli* and *D. polymorphus* suspensions (10⁶ CFU/ml) were dropped onto the prepared wafers and stayed at room temperature for 5, 10 and 20 min. *E. coli* and *D. polymorphus* were then washed with 500 μl PBS and spread onto LB and YPD agar plates, respectively. Both bacteria and yeast cultures of 48 h revealed no colony after treatment with Ag/Al₂O₃ for 5 min, but 16–19 yeast colonies were found using Cu/Al₂O₃. On the other hand, 10⁵–10⁶ colonies of *E. coli*

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and *D. polymorphus* were, respectively, detected in control tests with Al_2O_3 wafers and filter papers. The inactivation of *D. polymorphus* by the two catalysts was supported by light-microscopy observation. When the viability stain method with trypan blue was applied to catalytically inactivated yeast cells, a large number of strongly coloured cells appeared after treated for 5 min using $\text{Ag}/\text{Al}_2\text{O}_3$ and 10 min using $\text{Cu}/\text{Al}_2\text{O}_3$ in contrast to the control.

Figs. 1(a) and (b) show the scanning electron microscopy (SEM) images of yeast cells adsorbed on $\text{Ag}/\text{Al}_2\text{O}_3$ at room temperature in air for 5 min. The cell surfaces were densely covered with nanograde granules. Some of cells collapsed and led to release of inclusions (Fig. 1(b)). These SEM images show that the yeast cells are dramatically destructed on the $\text{Ag}/\text{Al}_2\text{O}_3$ surface,

which indicates that the inactivation is caused by chemical reaction and decomposition. However, Figs. 1(c) and (d) SEM images show that the cell surfaces were quite smooth on the $\text{Cu}/\text{Al}_2\text{O}_3$ wafer even though most of them were inactivated.

To see the eradication effect on SARS coronavirus, 10^6 PFU/ml TCID₅₀ viruses (100 μl) were loaded onto the wafers of $\text{Ag}/\text{Al}_2\text{O}_3$ and $\text{Cu}/\text{Al}_2\text{O}_3$ and the viral infectivity was measured in Vero cells. The eluted solutions of all the tested wafers after treatment for 5 and 20 min did not show detectable cytopathic effect (CPE) in Vero cells 48 h postinfection, whereas the eluted solution of the filter paper used as a control induced typical CPE.

In order to confirm the killing capacity to DNA viruses, 100 μl of 10^6 PFU/ml recombinant baculoviruses

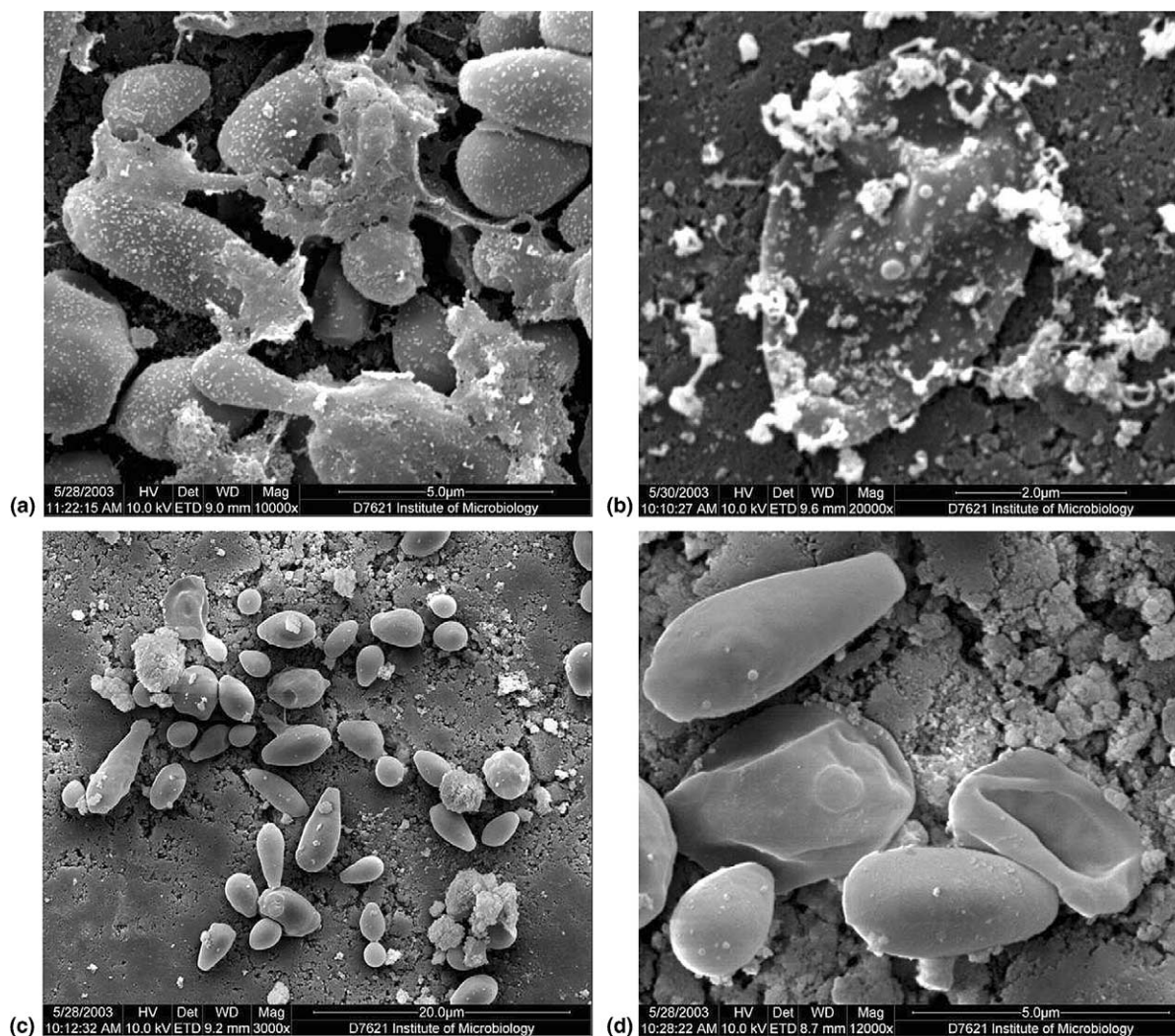


Fig. 1. SEM photograph of *D. polymorphus* on $\text{Ag}/\text{Al}_2\text{O}_3$ and $\text{Cu}/\text{Al}_2\text{O}_3$ wafers treated for 5 min. (a) 10,000 \times on $\text{Ag}/\text{Al}_2\text{O}_3$. (b) 20,000 \times on $\text{Ag}/\text{Al}_2\text{O}_3$. (c) 3000 \times on $\text{Cu}/\text{Al}_2\text{O}_3$. (d) 12,000 \times on $\text{Cu}/\text{Al}_2\text{O}_3$. 20 μl of 10^8 CFU/ml yeast cell was loaded on the surfaces of the wafers at room temperature in air. After 5 min, the wafers were fixed with glutaraldehyde and osmium tetroxide, drained with ethanol/water in increasing concentrations of ethanol. The absolute ethanol was replaced by dimethoxymethane, and the samples underwent critical point drying with CO_2 . The wafers were glued onto stages with conductive silver and metallized with gold. The samples were microscoped and photographed with a scanning electron microscope (Fei QUANTA 200).

Table 1
Inactivation efficiencies of different materials to various microorganisms

Materials	SARS coronavirus (CPE)	Baculovirus (CPE)	<i>E. coli</i> (CFU/ml)	<i>D. polymorphus</i> (CFU/ml)
Ag/Al ₂ O ₃	Undetectable ^a	Undetectable ^a	Undetectable ^a	Undetectable ^a
Cu/Al ₂ O ₃	Undetectable ^c	Undetectable ^c	Undetectable ^c	10 ^{2b}
Al ₂ O ₃	–	–	–	10 ^{5b}
Filter paper	Typical ^c	Typical ^c	10 ^{6c}	10 ^{5b}

“–”, not available. The dosage used is 100 µl of 10⁶ CPE/ml for SARS coronavirus and Baculovirus, 20 µl of 10⁸ CFU/ml for *E. coli* and *D. polymorphus*.

^a 5 min treatment.

^b 10 min treatment.

^c 20 min treatment.

that express hamster PrP protein [8] in insect cell Sf9 were loaded onto the Ag/Al₂O₃ wafer. No distinct CPE could be found in Sf9 cells using the elution of Ag/Al₂O₃ wafer treated for 5 and 20 min, respectively. Western blot assays with PrP specific monoclonal antibody revealed that the recombinant virus treated with Ag/Al₂O₃ wafer could not produce any detectable PrP protein. However, the elution of the control filter revealed the expected band. The results of inactivation to the three microbes are summarized in Table 1. In conclusion, the SARS coronavirus, bacteria and yeast are completely inactivated in 5 min on Ag catalyst surface and in 20 min on Cu catalyst surface at room temperature in air.

The mechanism of inactivation for these microbes on Ag/Al₂O₃ and Cu/Al₂O₃ surfaces is still not clear, but we can exclude the factor of dehydration, because Ag/Al₂O₃, Cu/Al₂O₃ and Al₂O₃ have a similar capability of water adsorption. One can also note that the remains of yeast cells on Ag/Al₂O₃ and Cu/Al₂O₃ are quite different, though they are all inactivated, indicating different mechanisms for the two catalysts. It is worth while to point out that both Ag/Al₂O₃ and Cu/Al₂O₃ surfaces did not show any disinfection for bacteria and yeast in a close environment without oxygen, which strongly in-

dicates that the inactivation is caused by catalytic oxidation rather than by toxicity of heavy metals.

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