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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/Al_2O_3 and Cu/Al_2O_3 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 (TiO_2) 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/Al_2O_3 and Cu/Al_2O_3 to SARS coronavirus, bacteria and yeast.

2. Experimental

The supported catalysts, Ag/Al_2O_3 (Ag 5 wt%) and Cu/Al_2O_3 (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/Al_2O_3, Cu/Al_2O_3 and $A1_2O_3$ powders were pressed into wafers of ca. 20 mg/cm^2.

3. Results and discussion

To address inactivation efficiency of Ag/Al_2O_3 and Cu/Al_2O_3, *Escherichia coli* and *D. polymorphus* suspensions (10^6 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/Al_2O_3 for 5 min, but 16–19 yeast colonies were found using Cu/Al_2O_3. On the other hand, 10^5–10^6 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 Ag/Al_2O_3 and 10 min using Cu/Al_2O_3 in contrast to the control.

Figs. 1(a) and (b) show the scanning electron microscopy (SEM) images of yeast cells adsorbed on Ag/Al_2O_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 Ag/Al_2O_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 Cu/Al_2O_3 wafer even though most of them were inactivated.

To see the eradication effect on SARS coronavirus, 10^6 PFU/ml $TCID_{50}$ viruses (100 μl) were loaded onto the wafers of Ag/Al_2O_3 and Cu/Al_2O_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

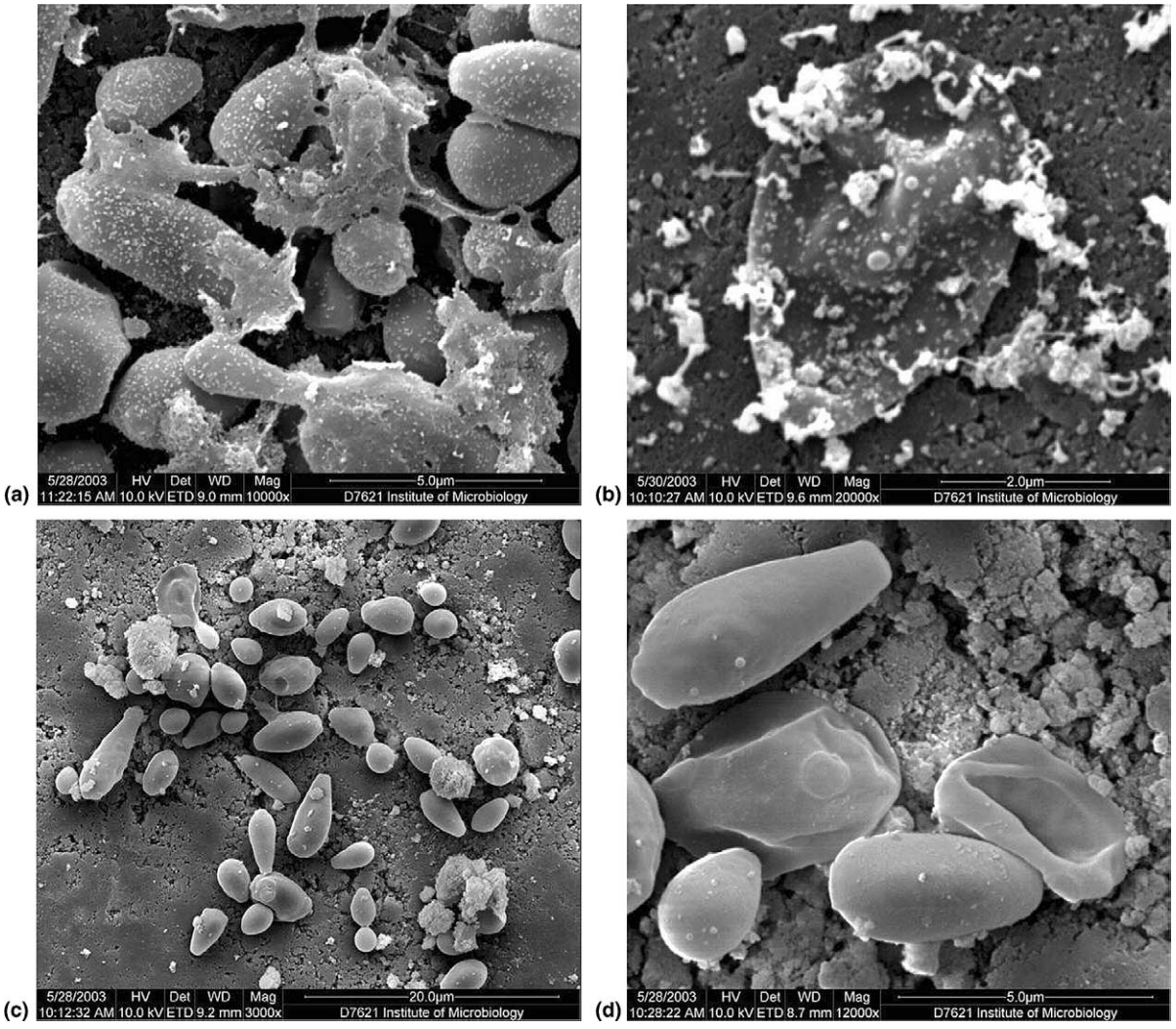

Fig. 1. SEM photograph of *D. polymorphus* on Ag/Al_2O_3 and Cu/Al_2O_3 wafers treated for 5 min. (a) 10,000× on Ag/Al_2O_3. (b) 20,000× on Ag/Al_2O_3. (c) 3000× on Cu/Al_2O_3. (d) 12,000× on Cu/Al_2O_3. 20 μl of 10^8 CFU/ml yeast cell was loaded on the surfaces of the wafers at room temperature in air. 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)	*E. coli* (CFU/ml)	*D. polymorphus* (CFU/ml)
Ag/Al_2O_3	Undetectable[a]	Undetectable[a]	Undetectable[a]	Undetectable[a]
Cu/Al_2O_3	Undetectable[c]	Undetectable[c]	Undetectable[c]	10^{2}[b]
Al_2O_3	–	–	–	10^{5}[b]
Filter paper	Typical[c]	Typical[c]	10^{6}[c]	10^{5}[b]

"–", not available. The dosage used is 100 μl of 10^6 CPE/ml for SARS coronavirus and Baculovirus, 20 μl of 10^8 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/Al_2O_3 wafer. No distinct CPE could be found in Sf9 cells using the elution of Ag/Al_2O_3 wafer treated for 5 and 20 min, respectively. Western blot assays with PrP specific monoclonal antibody revealed that the recombinant virus treated with Ag/Al_2O_3 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/Al_2O_3 and Cu/Al_2O_3 surfaces is still not clear, but we can exclude the factor of dehydration, because Ag/Al_2O_3, Cu/Al_2O_3 and Al_2O_3 have a similar capability of water adsorption. One can also note that the remains of yeast cells on Ag/Al_2O_3 and Cu/Al_2O_3 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/Al_2O_3 and Cu/Al_2O_3 surfaces did not show any disinfection for bacteria and yeast in a close environment without oxygen, which strongly indicates that the inactivation is caused by catalytic oxidation rather than by toxicity of heavy metals.

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