

The evaluation of activities of two types of photocatalysts at inactivation/disintegration of microorganism aerosols

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Photocatalytic processes are more and more widely used to remove different pollutants including microorganisms from liquid and gaseous media. However, methods for evaluating the efficiency of photocatalysts at inactivation/disintegration of microorganisms in the liquid film have limitations at predicting their efficiency for microorganism-containing aerosols. The comparison of two types of photocatalysts was performed on *Mycobacterium smegmatis* bacterium and vaccinia virus using these new methods for evaluating the efficiency of photocatalysts at inactivation/disintegration of microorganisms in aerosols deposited on photocatalytic coatings. It has been shown that the photocatalyst based on platinized TiO₂ displays a higher activity at inactivation/disintegration of aerosols of both microorganisms than the photocatalyst based on “pure” titanium dioxide.

1. Introduction

Photocatalytic processes are more and more widely used to remove molecular pollutants (Parmon, 1999; Shen and Ku, 2002; Sunada, et al., 1998; Vidal, et al., 1999) and suspended particles including different microorganisms (Kim, et al., 2006; Kuhn, et al., 2003; Lu, et al., 2003; Rincon, et al., 2001; Seven, et al., 2004; Sunada, et al., 1998; Sunada, et al., 2003; Vidal, et al., 1999; Vohra, et al., 2005; Zan, et al., 2007) from liquid and gaseous media. This is connected with the fact that photocatalytic devices can not only “remove” pollutants from air like usual adsorption filters do, but also destroy them (Kuhn, et al., 2003; Lu, et al., 2003; Sunada, et al., 2003; Robertson, et al., 2005). When pollutants are removed from a liquid medium, there is no shortage of water molecules, which are necessary for efficient photocatalytic oxidation of pollutants; there are also no limitations on the pollutant transfer to the photocatalytic site where the pollutant meets the forming radicals (Huang, et al., 2000; Nadtochenko, et al., 2006; Rincon, et al., 2001). The situation is different in the air for aerosol particles. Firstly, in

dry air there can be insufficient quantity of water molecules near the photocatalytic site, which will result in decreased production of radicals under irradiation. Secondly, the possibility that the pollutant inside the particle can meet the photocatalytic site is significantly lower as a result of a sharp increase in the particle viscosity at drying or in the case of a dry initial particle.

Methods to evaluate the activity of photocatalysts at inactivation/disintegration of microorganisms described in the literature mainly use thin liquid films containing microorganism suspensions deposited on the surface of a glass slide coated with a known amount of photocatalyst (Kuhn, et al., 2003; Sunada, et al., 1998; Sunada, et al., 2003). In nature, microorganism-containing aerosols are liquid only in the case of nearly 100% relative humidity, and if they have been initially liquid or are strongly hygroscopic. In other cases, microorganism-containing particles are a little dried or even absolutely dry. Therefore, the film application methods can be hardly used to analyze the photocatalysts' effect on microorganisms present in aerosol. More correct evaluation of photocatalytic activity at inactivation/disintegration of microorganisms present in deposited aerosol is possible by using a working air cleaner and microorganism aerosol passes through it. However, at the stage of selecting optimal photocatalysts for a concrete device, it is expedient to compare their photocatalytic activity at inactivation/disintegration of microorganisms in aerosol. This can be done when aerosols deposit on photocatalyst-coated glass slides and when they are on the surface under conditions of real relative humidity.

The present work is devoted to the comparison of the activities of two different photocatalysts at inactivation/disintegration of microorganisms according to a new technique.

2. Materials and methods

2.1 Experimental set-up

The proposed methods were implemented in a sealed chamber for sterile application of microorganism-containing aerosols to photocatalyst-coated and "pure" glass slides (Fig. 1). This chamber is a thick-walled glass cylinder with a cone attached on the top and four connecting pipes attached at the bottom. The cylinder dimensions are as follows: height - 280 mm, the inner diameter - 155 mm; the cone height - 200 mm. The working volume is 6544 cm³. A sprayer is screwed into the upper part of the cone. A grid with a diameter of 150 mm is placed at the height of 70 mm from the bottom inside the cylinder. The grid covered with Petryanov cloth to increase the evenness of distribution of aerosol mass deposited on the slides. Up to 12 slides were put upon the cloth. The dispersion system consists of two compressors connected in parallel (MOD 5KH33EN25T and MOD 5KH36KN1971 General Electric Inc., USA); a FV-1,6 filter; a Collision type pneumatic sprayer creating aerosol with a mean mass diameter of particles of 1 - 2 μm; and a syringe doser (HARVARD APPARATUS COMPACT INFUSION PUMP manufactured in the USA). Individual parts of the system are connected with rubber vacuum tubes. The compressed air pressure was controlled with an MTI-160 manometer, the temperature and relative humidity – with an "Iva-6M" thermohygrometer (manufactured by the Scientific and Production Company

“MICROPHOR”, Russia). The aerosol removal system consists of a measuring cell and a B-0,4 high performance aerosol filter connected with vacuum tubes.

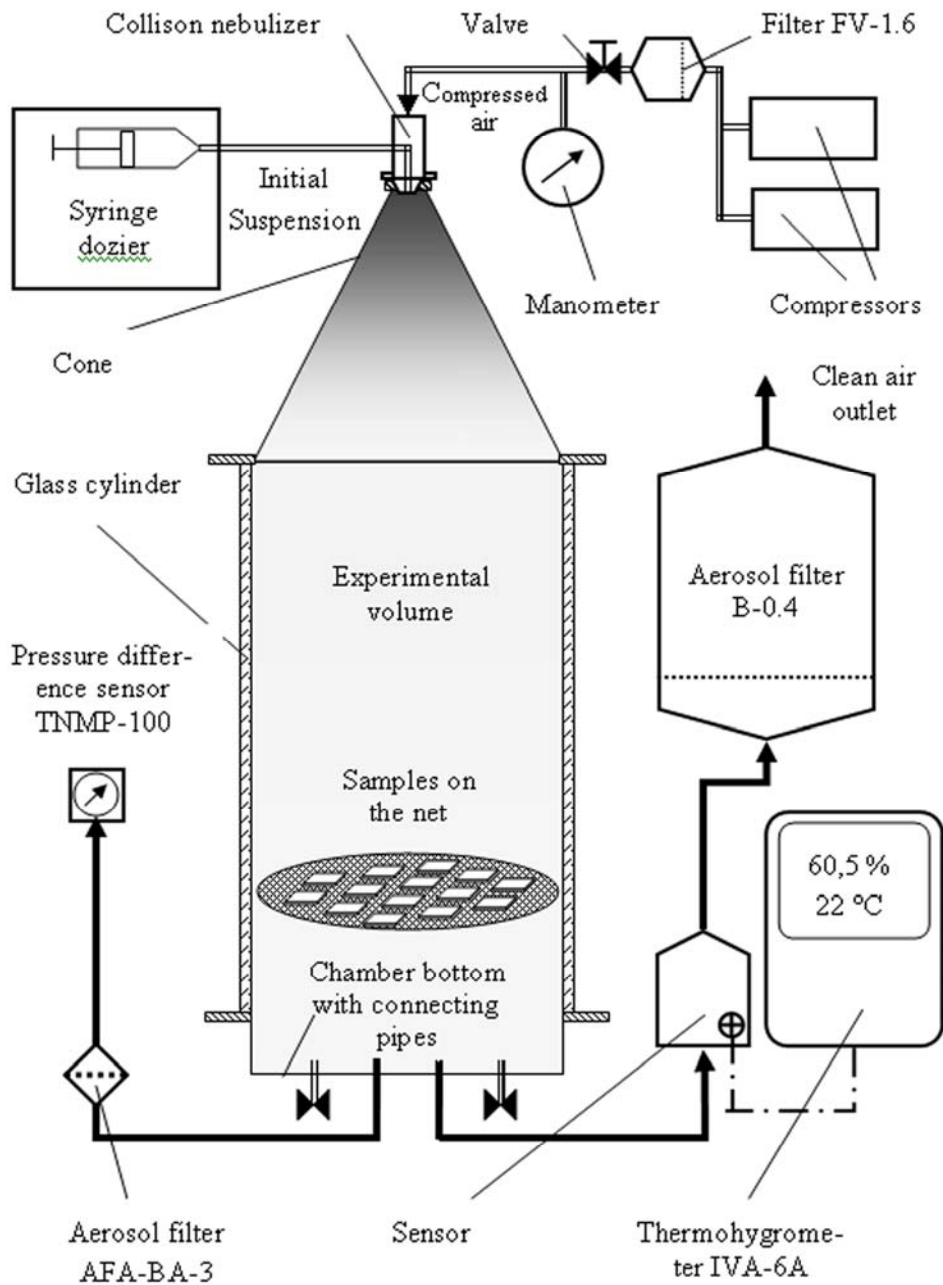


Fig. 1. The implementation scheme of the method for bioaerosol application to glass slides.

2.2 Photocatalysts

Slides with photocatalytic coating based on “pure” titanium dioxide or platinized titanium dioxide were prepared at the Boreskov Institute of Catalysis SB RAS. After applying to glasses, the coatings were subjected to sterilization at 110°C for 1 hour followed by washing in physiological solution.

2.3 Microorganisms

Both microorganisms were obtained from the Microorganism Collection of FSRI SRC VB Vector: bacterium *Mycobacterium smegmatis*, strain B-836; and vaccinia virus strain LIVP.

Mycobacterium smegmatis was grown in liquid LB medium at the temperature of 30°C on a thermostated shaker for 3 days. Ten-fold dilutions were prepared from the produced culture fluid, and 20 or 50 µl was seeded onto agarized FPA medium. The seedings were incubated in a thermostat at 30°C. The grown, isolated colonies were counted after 4-5 days of incubation. The results were calculated as the number of colony-forming units (CFU) per 1 ml of suspension. The biological activity of initial mycobacterial suspensions was $10^7 - 10^8$ CFU/ml.

Vaccinia virus was passaged 10 times on embryonated chicken eggs. Virus-containing material was produced by cultivation in cell culture 4647 followed by freezing/thawing the infected cell culture three times in supporting modified Eagle's medium (ICN Biomedicals, Inc., Aurora, Ohio). The standard method for counting the plaques forming on cell culture was used to determine the virus concentration in samples. The initial virus-containing suspension was diluted in supporting medium supplemented with antibiotics. Six 10-fold dilutions were prepared, and 100 µl was put onto a confluent monolayer of cells 4647 in 24-well plates (Costar, Pleasanton, California). The virus adsorption was carried out at 37°C for 1 hour in a CO₂ incubator. The plate was shaken every 10-15 minutes, the fluid was aspirated from the wells in 1 hour, and 2 ml of 1% agar (Difco) was added on RPMI 1640 medium (containing 2% fetal calf serum and antibiotics) to each well. Infected cells were incubated for 2 days at 37°C in a CO₂ incubator. Subsequently, the cell monolayer was stained with a dye prepared on the basis of gencyan violet to count the plaques in the wells. The results were calculated as the number of plaque-forming units (PFU) per 1 ml of suspension. The initial biological activity of viral suspensions was $10^7 - 10^8$ PFU /ml. Virus-containing suspension was frozen and stored at -70°C till it was used.

The samples' biological activity was determined in three parallel experiments. The calculations of the microorganisms' biological activities were performed according to methods described in (Ashmarin and Vorobyov, 1962).

2.4 Physical label

The uranin physical label was added to sprayed suspensions to evaluate the weight of aerosol deposited on glass slides. This dye is often used as a physical label at the study of microorganisms' survival under the influence of different factors (Ijaz, et al., 1985; Rehmenskii, 1973, p. 38; Sattar, et al., 1984). It was used further in all the conducted experiments. Uranin was sterilely added to the initial suspension to the final concentration of 10^{-5} g/ml. Fluorescence intensities of diluted samples proportional to concentration of initial material were measured with Spekol-11 spectrophotometer (Carl

Zeiss Jena, Germany) at uranin concentration lower than 10^{-7} g/ml, which provided a linear dependence of the label concentration on fluorescence intensity.

Experimental values of fluorescence intensities and biological activity of samples were used to calculate the efficiency of inactivation of selected biological agents.

2.5 Experiments on photoinactivation of microorganisms

Aerosol particles were deposited on 25*25 mm photocatalyst-coated glasses in the described sealed chamber. The duration of aerosol application was 3 – 5 minutes followed by drying for 27 - 25 minutes.

Exposure of samples to ultraviolet radiation was performed from 1 to 30 minutes with the lamp PHILIPS PL-S 11W/10/2P installed at the distance of 22 cm from the sample surface; the exposure rate of 0.65 ± 0.05 mW/cm² was measured with a radiometer (UVX digital radiometer, UVP Inc., USA). From 3 to 12 slides were irradiated simultaneously.

All experiments with photocatalyst samples and different controls were repeated three times.

3. Results and discussion

First of all, the degree of inactivation/disintegration of physical label was estimated under stricter conditions than those under which further experiments were conducted. An insignificant decrease in uranin amount was revealed at its application as a part of suspension non-containing microorganisms to photocatalytic coating at mild ultraviolet irradiation for 60 minutes with irradiance of 3.2 ± 0.4 mW/cm², which allowed using it as a physical label in further studies. It should be noted that a really achievable error of determination of the microorganisms' biological activity made up about ± 0.2 logarithm of the value, which significantly exceeds all the other experimental errors of measurements.

Then the toxic effect (without irradiation) of photocatalytic coating on selected microorganisms and their inactivation were evaluated at exposure to mild ultraviolet radiation. Series of control experiments with clean glasses at different exposures to ultraviolet radiation and non-irradiated photocatalyst-coated glasses were performed for this purpose. In addition, control experiments with clean glasses allowed us to obtain data on the natural inactivation of microorganisms under conditions of the experiment.

Unevenness of distribution of aerosol mass over glass slides being simultaneously in the sealed chamber where aerosol deposition took place, made up about 10%, which is a small value taking into account the accuracy of determination of biological concentration in samples.

Experimental data on the study of photocatalytic activity of two types of photocatalysts applied to glass slides at inactivation/disintegration of *Mycobacterium smegmatis* and vaccinia virus are summarized in the Table. Irradiated slides without photocatalytic coating and with applied microorganism aerosol served as a control. During 30 minutes, no losses of biological activity of aerosol-deposited microorganisms were recorded on non-irradiated slides with and without photocatalyst, that's why these results were not included in the Table.

Table. Data on the loss of biological activity of microorganism aerosol deposited on glass slides of (the portion of the initial biological activity) exposed at different periods of times to mild ultraviolet radiation according to the technique described in Section 2.5. An average of values measured in three repeats \pm their confidence intervals are given at the confidence level of 95%.

Irradiation time, min	<i>Mycobacterium smegmatis</i>				Vaccinia virus			
	Photocatalyst based on TiO ₂		Photocatalyst based on platinized TiO ₂		Photocatalyst based on TiO ₂		Photocatalyst based on platinized TiO ₂	
	sample	control	sample	control	sample	control	sample	control
0	0.0624 \pm 0.0099	0.0689 \pm 0.0083	0	0.0624 \pm 0.0099	0.0689 \pm 0.0083	0	0.0624 \pm 0.0099	0.0689 \pm 0.0083
1	0.0493 \pm 0.0069	-	1	0.0493 \pm 0.0069	-	1	0.0493 \pm 0.0069	-
10	0.0391 \pm 0.0031	-	10	0.0391 \pm 0.0031	-	10	0.0391 \pm 0.0031	-
20	0.0156 \pm 0.0030	-	20	0.0156 \pm 0.0030	-	20	0.0156 \pm 0.0030	-
30	0.0072 \pm 0.0014	0.0439 \pm 0.0048	30	0.0072 \pm 0.0014	0.0439 \pm 0.0048	30	0.0072 \pm 0.0014	0.0439 \pm 0.0048

Note: “-” – no data available.

A considerable photocatalytic activity was revealed for both types of photocatalysts applied to glass slides: the number of culturable bacteria decreased by 8.7 times within 30 minutes of irradiation when “pure” titanium dioxide was used and by more than 50 times when platinized titanium dioxide was used. The losses of activity of irradiated bacteria on glasses without photocatalytic coating during the same period made up 1.6 – 1.9 times. Therefore, within 30 minutes of irradiation, the number of culturable bacteria decreased by 5.6 times under the influence of “pure” titanium dioxide, and by more than 30 times – under the influence of platinized titanium dioxide. It should be noted that the obtained results are reliable as the estimated errors of measurements of the change in relative biological activity of microorganisms by the whole set of experiments usually did not exceed 1.6 times.

Similarly, under the same conditions for vaccinia virus, the amount of viable virus decreased by approximately 10 times under the influence of “pure” TiO₂, and under the influence of platinized TiO₂ – by more than 20 times, see the Table.

As follows from the above-said, the carried out work demonstrates the presence of inactivation/destruction of *Mycobacterium smegmatis* and vaccinia virus applied as aerosol to photocatalyst-coated slides at their exposure to UV radiation. Photocatalytic activity of catalysts at inactivation/disintegration of both microorganisms based on platinized titanium dioxide is by 2 – 5 times higher than that for “pure” titanium dioxide. Thus, there are all grounds to hope that these photocatalysts will be efficient also in real air-cleaners at inactivation/disintegration of captured bioaerosols.

4. Conclusion

The developed method of application of microorganism-containing aerosols to photocatalyst-coated glass slides in a sealed chamber allows us to perform a more correct evaluation of photocatalytic activity of the studied photocatalysts, to determine the loss of biological activity of the studied microorganism aerosols deposited on a support. The conducted experiments demonstrated the presence of considerable inactivation of microorganisms deposited as aerosol to photocatalyst-coated slides at their exposure to UV radiation. It has been shown that the photocatalyst based on platinized TiO₂ displays a higher activity at inactivation/disintegration aerosols of microorganisms than that the photocatalyst based on “pure” titanium dioxide.

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