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Non-UV based germicidal activity of metal-doped TiO_2 coating on solid surfaces

LIU Li-fen[1,2,*], John Barford[2], YEUNG King Lun[2], SI Grace[2]

1. School of Environmental and Biological Science and Technology, Dalian University of Technology, Dalian 116023, China. E-mail: kelifen@hotmail.com
2. Department of Chemical Engineering, Hong Kong University of Science and Technology, Clear Water Bay Road, Kowloon, HK SAR

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Abstract

A stain-based screening method was developed to screen different catalyst coatings for their germicidal activity. A Baclight dead/live bacteria viability kit (invitrogen, molecular probes) was used for staining the cell. The screening was carried out following a standard procedure. This included loading cell suspension to solid surface and maintaining contact for 30 min, then staining with a mixture containing dyes. The stained cells were observed using an epifluorescent microscope and photographed with a CCD camera under UV. Metal-doped TiO_2 coatings on Al plates were prepared and tested for non-UV germicidal activity without using UV. It was tested using model microorganisms such as Bakers Yeast (*Saccharomyces cerevisiae*), *Bacillus subtilis*, *Pseudomonas putida*, and *Escherichia coli*. On the basis of the germicidal activity of catalyst and the degree of damage caused to the cells, the stained cells may appear green (viable), green with red or yellow nuclei and yellow (compromised) or red (nonviable). According to their stained color, cells were counted to calculate the percentage of dead, live, and compromised cells. Compromised cells are cells that grow very slowly after reculturing indicating a degree of reversible cell damage. Screening the germicidal activity using this staining method is accurate and efficient, and requires less time than the culture-based method. A modification to the procedure for measuring germicidal activity of rough surfaces or fibrous coatings was developed. Both TiO_2 and metal-doped TiO_2 (Ag, Pt, Au, Cu) possess non-UV based germicidal activity. The germicidal activity of TiO_2 was found to be related with its wetting property and can be improved by UV irradiation before testing. It is not greatly affected by contact time, indicating a fast acting germicidal activity.

Key words: staining, germicidal activity, TiO_2 coating

Introduction

Concerns with indoor air bioaerosol levels and the spread of contagious diseases through touching surfaces like escalator handrails and lift buttons in public areas, has stimulated research on bioaerosol disinfection of air and surfaces. The commonly used disinfection methods include UV irradiation, fumigation, disinfection with chemicals and filtering. The first two methods have limited direct use in public areas like railway stations, subways, hospital waiting rooms, etc., where it is impractical to apply them during normal operation when people are present. Cleaning the surfaces by disinfection is easier, but has risks from possible harmful by-product pollution. Bioaerosols may be trapped in filters and/or accumulate on surfaces within air conditioners or air purifiers, where the surfaces may be used for bioaerosol disinfection and mineralization by applying highly active nanocatalyst coatings. Disinfections of bioaerosol by a non-UV based germicidal material coating are proposed by researchers in Hong Kong University of Science and Technology (HKUST). The nanomaterial coating can be used for bioaerosol disinfection when incorporated into an air treatment unit. Yan *et al*. (2005) reported a high germicidal activity of Ag/Al_2O_3 in the absence of UV, which may not be caused by the toxic Ag^+. Ag promoted the transformation of oxygen molecules to reactive oxygen species (ROS), and the oxidation caused damage to cell membranes. Kuhn from Germany (2003) first used TiO_2/ UVA in disinfecting surfaces. They inoculated a sample onto an agar plate and counted the colony forming units. Trypan Blue was used to determine the viability of the cells. Viable cells appeared light blue after staining, whereas dead cells appeared deep blue.

The measurement of the germicidal activity of coated material on a surface and its effectiveness over a certain time in an air treatment unit meet several practical problems. Traditional incubation methods have often been used, but it takes a long time and it maybe inaccurate when the bacteria are compromised by dehydration, starvation, and other stress factors. Because it may be difficult to revive and the rate of reproduction maybe low during culturing (Tsai *et al*., 2002). The CFU (colony forming

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*Corresponding author. E-mail: kelifen@hotmail.com.

unit) method measures viable cells and cells that are culturable in a reasonable period of time (1–3 d).

Methods for staining the bacterial cells to measure the viability have been widely used in biological studies. Different fluorophores (Beloti and Barros, 1999; Gavin *et al*., 2000; Mason, 1993) e.g. Acridine orange, TTC (2,3,5-triphenyl-2-tetrazolium chloride), Hoechest 33258, FUN-1 family (Millard *et al*., 1997), methylene blue and Baclight Dead/Live Bacteria Viability Kits (based on Syto-9 dye and Propidium Iodide, Invitrogen Product from Molecular Probes, USA, have been used for this purpose. These can be combined with flow-cytometry (Lange *et al*., 2001), confocal laser fluorescent microscopy (Millard *et al*., 1997), or simply epi-fluorescent microscopy. For microscopic analysis and cell viability tests, it is better to use nonfluorescent dyes. Although acridine orange binds to DNA imparting a red color within the cell, inconsistent results may be obtained when cells are in different physiological activity (McTeters *et al*., 1991). This makes it difficult to interpret the viability of cells that are not fully stained. And they may require a high concentration of cells and the color change after staining may require longer time to appear e.g. TTC (Beloti and Barros, 1999). And they may require a fixation step and a washing step that may introduce errors in estimations of viability (e.g. Methylene Blue). The only disadvantage of staining method is the toxic and mutagenic property of dyes. Genetically modified strains, e.g. *E. coli*, in which a green fluorescent protein gene have been inserted, give fluorescence (possibly requiring induction with acyl-homoserine lactone and/or antibiotics) only in viable cells. However, it may be difficult to insert the right gene into the cell of selected test microorganisms and to relate its fluorescence to the state of viable cells (Webbs *et al*., 2001). These methods, however, offer a harmless and more convenient way for screening germicidal activity of catalyst by simply counting the number of cells on the catalyst surface where the green fluorescence has not been extinguished.

Pathogenic microorganisms are often used for testing the germicidal activity of materials. These, however, require stringent biosafety precautions. For screening in a laboratory that only with Biosafety Level 1 standard, Yeast is a convenient choice, which is often used as a model microorganism in many research activities. Its viability measurement has been investigated with many fluorophores (Van Zandy *et al*., 2003). It has an advantage over other bacteria for its large cell size and the clarity in the pictures of stained cells observed under microscope.

1 Methods and materials

1.1 Test microorganisms and cell suspension preparation

Microorganisms were cultivated overnight at 30°C in an incubator (*Bacillius subtilis* on TSA media, *Saccharomyces cerevisiae* on modified malt extract media and *Pseudomonas putida* on LB). Agar plate containing these cultures were kept in refrigerator and were regularly subcultured. Cell colonies from an agar plate was swabbed and resuspended in saline solution (0.8%) to the desired optical density (OD) value. The cell count was measured using a Coulter Multisizer II.

1.2 Cell loading and contacting with catalyst on Al surface

A cell suspension was loaded to coated Al plate (1.8 cm×1.8 cm) using a pipette. They were allowed to contact with the surface coating on the Al plate for 30 min. A blank aluminum plate was also loaded and was used as a control. Initial viability of cell suspension was measured by loading the cell suspension onto a glass slide. The loaded cell sample may be dried within 2 min with airflow over the surface of plate. The blank plate, tested with 1 h contacting with the cells, showed no germicidal effect.

1.3 Staining

After the required contacting time, if the surface is smooth, cell viability can be measured by adding staining solution directly on the surface. If the surface is rough or fiberous, the cells are removed from the surface by washing and swabbing and then recovered using a polycarbonate filter (Osmonics, 20 mm diameter). The staining solution was then added. Microscopy with 100X magnification was then used to observe the stained cells on the surface or the recovered and stained cells on the polycarbonate filter. Pictures were taken of fluorescent cells using microscope and CCD camera (Olympus, Japan) under UV, using filter 2. The pictures were taken across the plate in a standardized way (from side to side and from bottom to top) to ensure statistically acceptable counts. A total of 20 to 30 pictures were taken to allow a high accuracy and a high confidential level (95%). Cell viability after contacting with catalyst surface was used as an indicator of germicidal activity.

The germicidal activity (%) of catalyst was defined as the change of viability of loaded cells as: (initial viability-final viability)/initial viability×100.

1.4 Baclight dead/live bacteria viability kits

Baclight dead/live bacteria viability staining kits (Introvigen: Catalog No.7012 or 13152 from Molecular Probes website) consist of two dye components Propidium Iodide (PI) and Syto 9. Propidium Iodide is a dye that can enter a cell only with a damaged cell membrane. It binds with RNA or DNA and stains the cell red. Syto 9 is a dye that stains cell membrane whether it is viable or nonviable and makes the cell appear green under fluorescence. The staining kit was used in accordance with the manufacturers instructions and with a 1:1 volume ratio of Syto 9 to PI solution, the concentration of PI is 12 mmol/L and Syto 9 is 2–3 mmol/L. The volume of staining solution used was 6 μl/ml cell suspension.

1.5 Germicidal coating on Al plate

Metal-doped TiO_2 coatings on Al plate were prepared by a sol-gel and dip-coating method or by a spin-coating method.

A total of 1 mol/L of titanium isopropoxide (ACROS) and isopropanol solution (BDH) was slowly added to deionized and distilled (DDI) water under vigorous stirring. The resulting solution was stirred for about 1 h to ensure complete hydrolysis of titanium isopropoxide. A desired amount of nitric acid (1.0 mol/L, $C_{H^+}/C_{Ti^{4+}}=0.4$) was then added to peptize the solution. The temperature was increased gradually to 70°C and was then maintained for 2 h to ensure the evaporation of the isopropanol. Subsequently, it was kept being stirred at room temperature until a clear TiO_2 sol (0.28 mol/L) was formed. Appropriate amount of $HAuCl_4$ (0.01 mol/L), $AgNO_3$ (0.05 mol/L), H_2PtCl_6 (0.01 mol/L) and $Cu(NO_3)_2$ (0.2 mol/L) precursor solutions were then added to this TiO_2 sol, respectively, to obtain a desired metal loading (0.5 wt%). A coating paste could be prepared by adding of 5 wt% of polyethylene glycol (PEG) with an average molecular weight of 400 g/mol (ACROS) into the metal-modified TiO_2 sol and by following drying step in an oven at 65°C.

In the present study, the aluminum plate (1.8 cm×1.8 cm) was used as a substrate. Before the coating, all the substrates were ultrasonically cleaned in the DDI water and acetone, respectively, with several cycles to remove the surface contamination and were dried in the oven. The metal (Ag, Au, Pt and Cu) doped TiO_2 coating aluminum plates were then successfully obtained by directly brushing the metal containing TiO_2 paste on the aluminum surface evenly and by following calcinations at 450°C for 1 h.

The catalyst was tested within 30 min when it was still fresh. Or else, the plates were sealed in a petridish until testing.

The germicidal activity decreases upon aging and upon exposure to air and this is the topic of further research.

1.6 Standard testing procedure with Yeast

Yeast was subcultured on MMA (modified malt extract agar plate) and then incubated for 16–24 h at 30°C. It was then stored in refrigerator at 4°C. The plate was taken out from the fridge, and the microorganism was stimulated for approximately 20 min before preparation of the suspension.

A cell suspension was prepared using sterilized 0.8% saline. The concentration of cell in suspension was adjusted to make its OD around 0.6–0.7. It was then stained for 10 min to ensure a high initial viability (>95%).

The catalyst plate was washed with sterilized water or 0.1% saline. A total of 1 ml was used each time, two to four times.

The washed plate was left on a glass slide in the Biosafety Cabinet to dry.

The catalyst plates were loaded (10–15 or 5–20 microliter). Individual plates were loaded at an interval of 5 min for 30 min. If more samples were required, another batch was prepared from another freshly prepared cell suspension. The contact time was 30 min.

A staining solution (1:1 volume ratio for two dye components, for Kit 7012 or 13152.) of equal volume as that of the loaded cell suspension was added to the loaded cell suspension. It was stained for 15 min.

The cells were observed using microscope. Preferentially two series of pictures were taken across the loaded spot (1 series of approximately 8–12 pictures).

The pictures were counted according to the counting criteria below average. The average was taken from the counting result and the error range (CI 95%) was calculated.

1.7 Criteria for counting/differentiating cells

Green, yellow, and red cells were counted according to fixed criteria to reduce subjective errors. The counting criteria for the stained cells pictures were: (1) a red cell is counted as a red cell; (2) a deep/dark orange cell is also counted as a red cell; (3) a yellow cell with 2/3 or more of the cell area visible being red/deep orange is counted as a red cell; (4) a green cell with no other colored spot inside the cell is counted as a green cell; (5) a green cell with a red spot is counted as a yellow cell; (6) light yellow and/or yellow cells are counted as yellow cells; (7) a yellow cell with less than 1/3 of the cell area visible being red is counted as a yellow cell.

2 Results

2.1 Staining of cells with Baclight Dead/Live Bacteria Viability Kits

This kit offers a mixture of dyes with no background fluorescence. Thus, staining on the surface allows direct microscopic observation of the metabolic state of cell after contact with the catalyst. Yeast cells treated with 70% ethanol appeared red (dead). Cells treated with a lower concentration of ethanol appeared compromised and appeared yellow, or green with yellow dots. Fresh live cells appeared green after staining. Overnight incubated fresh yeast cells were approximately 98% viable.

The catalytic germicidal activity reduces viable cells to different percentages of dead cells (stained red) and compromised cells (stained yellow). Good coating germicidal material (catalyst) can reduce the viability from more than 90% to less than 10% within 30 min. Cells that are stained yellow or green with red nuclei have an intermediate degree of damage, with a compromised membrane that allows some PI to enter the cell and to combine with the DNA or RNA to produce a red color. The Syto 9 dye combines with all cell parts without differentiation, and produces a green color. The extent to which the two dyes combine to cell material determines the overall color of the cell. Disinfecting yeast cells with 40% ethanol for 23 h, or 50% ethanol for approximately 50 min largely resulted in the production of compromised cells. These cells were stained and appeared yellow. Agar plates were inoculated with these types of cells and incubated at 30°C for 23 h. After incubation with 40% ethanol, tiny transparent colonies were formed after 2 d. These colonies did not grow further. Stained yellow cells from 50 min incubation with 50% ethanol required 6 d to form numerous tiny transparent colonies and three small milky colonies (diluted 1/10, 20 μl).

Yeast cells that were green with red dots can be incubated on an agar plate to form colonies. Normally, only a single day is required for normal yeast colonies to appear on an agar plate. Stained green cells with slight yellow dots grow better under incubation and may form normal colonies within 1–2 d.

The germicidal effect of the catalyst reduces the viability of yeast after a certain period of contact time. On the basis of experiment, the contact time was set at 30 min. Staining cells on the surface takes another 15 min.

When loading volume is around 5 to 10 ml, the cells will spread across the plate depending on the wetting property of the surface. Later, after the addition of the staining solution and cover slip, each picture with 100x magnification will contain approximately 50–150 yeast cells and more *P. putida* and/or *E. coli* cells. Approximately 20 pictures were taken across the cover slip in a standard method to reduce the statistical errors. The average percentage of cells normally has an accuracy range of ±2%–10% (CI 95%).

2.2 Germicidal activity of catalyst measured using different strains

2.2.1 Catalyst germicidal activity on *Bacillus*

The germicidal activity of TiO_2 on *Bacillus* is given in Table 1.

Low initial viability of *Bacillus* is possibly because of the relatively long growth compared to its doubling time and/or cells in different metabolic states appear different in size and form, thus causing inaccurate counting. The loading volume was 20 ml with an OD of 0.48. The germicidal activity of tested pure TiO_2 plate (No 14) is 55%. Counting *Bacillius* cells is difficult because of the cell form and presence of spores. Using other bacteria with more regular cell size and form will result in better accuracy.

2.2.2 Germicidal activity of TiO_2 on Bakers Yeast

Yeast has many advantages due to its relatively constant and large cell size and the distinctive staining patterns with the dye kit. For this reason, *S. cerevisiae* (separated from Bakers Yeast sachet for home use) was used. The same catalyst plate tested with yeast after 30 min contact showed 87% germicidal activity. Distribution of the cells according to stained color was: (13.41±4.1)% green, (52.73±7.4)% yellow, and (34.15±8.48)% red as shown in Table 2. The starting viability of yeast cells is approximately 95%, and the cells are stable for more than 60 min on blank Al plate.

Table 1 Cell distribution and germicidal activity of TiO_2 on *Bacillus*

Sample	Viable (green)	Compromised (yellow/red)
Initial cells	58%	42%
After 30 min contact with coating	26%	74%

Table 2 Germicidal activity of pure TiO_2 on *S. cerevisiae*

	Green (%)	Yellow (%)	Red (%)
Initial cells	95%		
After 30 min contact with TiO_2	13.41±4.1	52.73±7.4	34.15±8.5

2.2.3 Test with *Pseudomonas putida* on Ag/TiO_2

S. cerevisiae and *P. putida* were loaded separately on the Al plates that were coated with Ag-doped TiO_2 and were then tested for germicidal activity. The results are presented in Table 3. It showed that Ag-doped TiO_2 has a high germicidal activity. Ag often appears as mixtures of metal atom and ions as shown by many researchers, it may be leached out after loading and may contribute for the germicidal activity (Yan *et al*., 2005) besides that of pure TiO_2.

Blank plate test result for yeast shows approximately 94.5% viable cells. Blank test on Al for Ps putida has approximately 89% viable cells. B1 plate had higher percentage of red cell than B3 plates because B3 was loaded after B1. Although the yeast percentage affected by catalyst is similar, but the yellow cell on B3 is higher than B1, and red cell is lower than B1. The drop of complete killing activity of B3 from B1 was related to storage that resulted in decrease of activity. The germicidal activity is higher for *P. putida* than for *S. cerevisiae*.

2.3 Germicidal activities of different catalysts

2.3.1 Pt- and Cu-doped TiO_2

Pt- and Cu-doped TiO_2 coatings were tested using *S. cerevisiae*. The results are presented in Table 4.

These results showed that coatings of Pt- or Cu-doped TiO_2 can affect a high percentage of cells, but within 30 min the completely dead cells ratio is low compared with Ag-doped TiO_2, except for one plate. These metal-doped catalysts did not display a higher germicidal effect than Ag-doped TiO_2.

Catalyst plates were tested within 30 min after preparation or were stored in sealed petridish until testing. The loading sequence may affect the germicidal activity, because during the loading interval the catalyst was ex-

Table 3 Germicidal activity of Ag/TiO_2 on *Saccharomyces cerevisiae* and *Pseudomonas putida*

Catalyst No.	Model microorganism	Living cell (%)	Yellow cell (%)	Red cell (%)	Germicidal activity (%)
B_1 (Ag/TiO_2)	Baker's Yeast	9.78±5	54.02±8.7	36.19±8.5	90
	P. Putida	15.17±2	0	84.83±2	83.7
B_3 (Ag/TiO_2)	Baker's Yeast	10.46±4.4	76.82±9.6	12.71±7.0	90
	P. putida	2.51±2.17	57.61±17.7	39.86±18.0	97.8
Average	Bakers Yeast	10.2	65.4	24.4	90
	P. Putida	8.8	28.8	62	90
Initial viability	Yeast	94.5			
	P. putida	89			

Table 4 Yeast viability after 30 min contact with catalyst Pt/TiO_2 or Cu/TiO_2

Catalyst No.	Green cell (%)	Yellow cell (%)	Red cell (%)	Germicidal activity (%)
A1 (Pt/TiO_2)	6.71±3.6	77.34±6.2	15.94±3.7	95
	2.5±1.4	94.6±1.8	2.9±2.2	98
A2 (Pt/TiO_2)	10.73±3.16	75.35±5.51	13.91±3.09	88
	6.80±3.8	60.36±16.9	32.84±14.4	93
A3 (Cu/TiO_2)	17.03±3.6	79.61±3.9	3.34±1.4	83
	1.7±1.7	89.2±3.0	9.0±2.4	98
A4 (Cu/TiO_2)	9.55±3.92	76.68±5.73	13.76±3.87	88
No.45	23.17±4.76	65.58±6.55	11.24±5.20	73
No.49	18.53±3.2	67.62±6.1	13.85±4.1	82
Initial cells	98.5			

No. 45 and No. 49 are catalysts coated with Cu/TiO_2.

Table 5 Germicidal activity of coatings from Au-doped TiO_2

Catalyst Au/TiO_2	Green cell (%)	Yellow cell (%)	Red cell (%)	Germicidal activity* (%)
No.28	9.53±3.8	54.35±6.8	36.12±8.4	90
No.29	7.6±6.4	73.5±7.4	18.8±5.0	92.7
No.30	14.8± 4.5	74.4±5.3	10.8±3.4	85.5
No.46	15.43± 5.6	61.17±17	23.40±14.0	85
No.47	9.41±4.9	68.43±8.9	22.15±7.9	91
Initial cells	98.5			

No. 28–47 are catalysts coated with Au/TiO_2.

Table 6 Germicidal activity of catalyst C (pure TiO_2) series (on yeast)

Catalyst C series	Contact time (min)	Living cell (%)	Yellow cell (%)	Red cell (%)	Germicidal activity (%)
C_0	15	2.78±1.1	27.00±4.4	65.21±5.3	96
C_1	30	30.68±5.6	49.81±6.8	19.51±5.4	69
C_2	30	27.87±5.6	48.82±6.2	23.31±7.7	72
$C_{3\text{-}1}$	30	28.98±5.5	53.16±6.9	17.86±3.8	71
$C_{3\text{-}2}$	30	38.13±6.8	40.78±7.7	21.10±6.4	62
Average	30	31.41±5.4	48.14±6.2	20.4 ±2.7	70
C_4	15	30.35±5.0	38.13±5.8	31.51±3.3	70
C_4	23	13.94±4.3	57.00±5.7	29.05±3.8	86
Initial cells		98			

C: pure TiO_2

posed to air. Absorption of VOC may adversely affect the germicidal activity. Au-doped TiO_2 had better germicidal activity than Pt- or Cu-doped TiO_2, as indicated by the higher red cell percentage in Table 5.

2.3.2 Other tests on factors influencing germicidal activity: contact time or wetting property

The results of germicidal activity of C (pure TiO_2) and D (90% TiO_2-SiO_2) series are presented in Tables 6, 7, and 8. The aim was to investigate whether the activity of coating on different plates was related to its preparation and whether the contact time influences the germicidal activity in shorter contact time range.

The blank for this series of test has 98.5% viable cells. As can be seen in Table 6 : (1) the germicidal activity of the coating on different plate was similar; (2) the measurement for screening germicidal activity is reliable; (3) the highest germicidal activity was detected when the contact time was 15 min. It seems that there are other factors, which are more influential on the germicidal activity than contact time. This led to the discovery that germicidal activity related with superhydrophilic property of titanium based catalyst material and the oxidation property of defect sites where relative high concentration of oxygen vacancy or Ti^{3+} exists. This is the subject of a further report, with investigation of the mechanism and germicidal activity of the different catalyst by different analysis instruments.

The results of series tests on catalyst D (90% TiO_2-SiO_2) are listed in Table 7 and 8. It can be seen from Table 7 that 90% TiO_2-SiO_2 catalyst (D series test) with a contact time of 15 min or 30 min had similar germicidal activity.

After storage, the wetting property as well as the germicidal activity diminished as shown in Table 8. It was found that the germicidal activity was affected by the wetting property of surface coating. The germicidal activity of D25 was lost when it was stored in air for some time. D26 was UV irradiated for 15 min and was kept in the dark for 5 min before loading samples: UV irradiation improved wetting and also improved the germicidal activity.

2.4 Method modification

This method is reliable, repeatable, and provides an accurate measure of germicidal activity. It requires short period of time to complete and is efficient. It is also economic because it does not require complicated expensive instruments such as flow cytometry. Many other organisms can be used to test the germicidal activities of the materials.

This screening method can also be used in screening

Table 7 Germicidal activity of catalyst D (90% TiO_2-SiO_2) series (prepared by spin-coating)

Sample D	Contact time (min)	Green cell (%)	Yellow cell (%)	Red cell (%)	Germicidal activity (%)
D_{1-2}	15*	1.94±1.07	69.46±5.32	28.59±5.24	98
D_{1-1}	15*	0.76±1.4	72.87±4.6	26.3 ±4.1	99
D_2	30	2.00±2.25	67.89±3.20	24.83±3.15	97.7
D_3	30	6.14±2.66	67.35±6.17	26.52±5.32	92.9
D_4	30	9.81±2.50	73.09±4.41	17.10±4.63	88.6
Average		6.0	69.6	22.7	93.1
Initial cell		90			

Table 8 Effect of UV improved wetting property on germicidal activity of catalyst D (90% TiO_2-SiO_2)

Sample	Green cell (%)	Yellow cell (%)	Red cell (%)	Germicidal activity (%)
D25	97.1	0	2.3	0.5%
D26	5.90±3.7	92.80±4.2	1.30±1.0	94%
Initial cells	97.6			

germicidal materials that is not water soluble, such as fiber material, surface coating material with some modification of the procedures. For smooth coatings, the staining can be carried out directly on the surface. For coarse/uneven materials, the cells after exposure can be recovered in a solution, and the staining can be carried out in solution. This is followed by a filtering step to recover the stained cell in suspension using a black polycarbonate membrane. The filter can be then used for microscopis check up and to take pictures. The viability change of cells can be obtained by counting the viable cells and total cell according to their color using the defined criteria.

3 Conclusions

A fast, accurate, and reliable screening procedure has been established for measuring the germicidal activity of nanocatalysts coated on Al plate. This used Saccharomyces cerevisiae as the test organism. Damaged cells on the catalyst were clearly shown by their color of cells after staining. The intact viable cells are stained green, the compromised cells may appear green with red/yellow dots or yellow according to the degree of damage. Dead cells appear red. Whole yellow cells are severely damaged and require 6 d for the colony to appear on agar plate. This indicates that they are only capable of slow growth and have been severely damaged. Red cells are dead cells that have lost their ability to reproduce.

Catalyst coating from TiO_2 or metal-doped TiO_2 possess germicidal activities to microorganism without using UV. This germicidal activity is stronger when the coating has good wetting property improved by pretesting UV irradiation. The contact time did not significantly affect the germicidal activity. Ag doped TiO_2 has higher germicidal activity than other metal doped catalyst.

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