Customer Name	Maeda-Kougyou Japan 218-2 Norimatsu Yahatanishi ward Kitakyushu City Japan
Contact	Dr. Khaled Hussein
Customer PO no.	N/A
Test Requested	BS ISO 27447: 2009 Fine ceramics (advanced ceramics, advanced technical ceramics) — Test method for antibacterial activity of semiconducting photocatalytic materials (Test method modified due to client's requests)
Sample Description	Stainless steel: non-coated and coated with Miracle Titanium (Primary and MVX), Clear plastic: non-coated and coated with Miracle Titanium (Primary and MVX), Textile: non-coated and coated with Miracle Titanium (Primary and MVX)
Date of Receipt	27th April 2012
Project Number	ASCR0092007
Report Date	5th July 2012

Contents

Description of Test Items

The following items were tested. The samples to be tested all measured 50 x 50 mm.

Test Item	Product Code
Treated Stainless Steel	ASC002162
Untreated Stainless Steel	ASC002163
Treated Clear Plastic	ASC002164
Untreated Clear Plastic	ASC002165
Treated Textile	ASC002166
Untreated Textile	ASC002167

Introduction

The purpose of the project was to ascertain the effect of the MVX coating on bacterial viability when applied to stainless steel, plastic and textile following exposure to U.V and incandescent light as per the client's request. The test was agreed to be performed in accordance with BS ISO 27447: 2009 Fine ceramics (advanced ceramics, advanced technical ceramics) — Test method for antibacterial activity of semiconducting photocatalytic materials. As the client's requests included alterations in the type of materials to be tested, some alterations were made to the test method; however these have been subsequently noted in this report.

Procedure

The experimental procedure was performed in accordance with BS ISO 27447: 2009 Fine ceramics (advanced ceramics, advanced technical ceramics) — Test method for antibacterial activity of semiconducting photocatalytic materials, with alterations made to accommodate the client's sample and microbiological challenge specifications. The method for analysis was as follows:

<u>Modules 1 and 2: Stainless steel and Plastic (Film adhesion method)</u>

1. Cultures of *Escherichia coli* ATCC8739 were grown under aerobic conditions at 37°C for 18 ± 1 hours. The concentration of bacterial cells was adjusted to a target concentration of 2.6 x 10^6 cells ml^{-1} in 1/500 nutrient broth. The adjustment in cellular concentration was calculated from previously performing serial dilutions of an overnight culture and

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correlating the bacterial concentration against the level of absorbance at an optical density of 600 nm using a spectrophometer.

2. Prior to bacterial inoculation, all of the samples were surface sterilised with 70% (v/v) ethanol and were selected at random.
3. Specimens were individually placed in sterile petri-dishes. The specimens were placed on top of glass slides that separated the sample from the sterile wet filter paper, which was used as a moisture control measure.
4. A 150 µl aliquot of bacterial suspension was placed on top of the samples and immediately covered with sterilised film.
5. Treated and untreated samples were kept in a dark place or exposed to the light source as specified in the standard with the additional incandescent light source as per the client's request.
6. Three untreated samples were immediately withdrawn at t = 0 to ascertain the recovery of bacteria immediately after inoculation. Bacteria were extracted by washing in 10 ml Tryptic Soy Broth 0.05% (v/v) Tween-80.
7. A 1 ml aliquot of the washout was withdrawn and was serially diluted in phosphate buffered saline (PBS).
8. A 200 µl aliquot of the neat washout and serial dilutions was placed in a sterile petri dish. Approximately 15 – 20 ml Tryptic Soy Agar was added in order to enumerate viable cells by the pour plate method.
9. The solidified plates were allowed to set at room temperature and were incubated overnight at 37 ± 1°C.
10. Following incubation the agar plates were counted for the presence of colony forming units (cfu) and the results were recorded. Where the number of cfu exceeded 300 the plates were recorded as TNTC (Too numerous to count).

<u>Module 3: Textile Samples (Glass adhesion method)</u>

1. Prior to inoculation all samples and coverslips were sterilised by autoclaving at 121°C for 15 minutes.
2. A culture of *E. coli* ATCC3799 was grown in Tryptic Soy Broth under aerobic conditions at 37°C for 18 ± 1 hours.
3. The concentration of bacteria was adjusted to a target concentration of 1 x 10^5 cells ml^{-1} using 1/500 nutrient broth.

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4. Specimens were individually placed in sterile petri dishes. In order to preserve moisture a sterile filter paper was moistened with sterile water with the specimens to be tested separated by a glass slide.
5. A 150 µl aliquot of the adjusted bacterial suspension was placed on the surface of the textile samples and a glass slide was placed on top to press the bacterial suspension uniformly under the glass.
6. Treated and untreated samples were kept in a dark place or exposed to the light source as specified in the standard with the additional incandescent light source as per the client's request.
7. Three untreated samples were immediately washed in 20 ml PBS. A 2 ml aliquot of this washout was serially diluted in sterile PBS.
8. A 500 µl aliquot of the neat washout and the serially diluted samples was plated in duplicate on sterile petri-dishes.
9. Approximately 15 – 20 ml of Tryptic Soy Agar was placed into each petri dish in order to enumerate viable colony forming units by the pour plate method following incubation at 37°C for 24 – 48 hours. Where the number of cfu exceeded 300 the plates were recorded as TNTC (Too numerous to count).

Satisfaction of criteria for a valid test and calculations

The test requirement fulfilment validation follows the raw data in the results section (see below). In addition the results expressing photocatalyst antibacterial activity value for hard surfaces (R_L) and on textiles (S_L) and the photocatalyst antibacterial activity value with UV and incandescent light irradiation for hard surfaces (ΔR) and on textiles (ΔS).

Film adhesion method

$N = P \times V$

N is the number of viable bacteria

P is the bacteria concentration (cells/ml)

V is the volume of extraction buffer used in the test

1. The logarithmic value of the number of viable bacteria of non-treated specimens after inoculation is:

$(L_{max} - L_{min})/(L_{mean}) < 0.2$

L_{max} is the maximum logarithmic value of viable bacteria

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L_{min} is the maximum logarithmic value of viable bacteria

L_{mean} is the average logarithmic value of viable bacteria for 3 specimens

2. The logarithmic value of viable bacteria of non-treated specimens after inoculation shall be within the 1.0×10^5 to 4.0×10^5 range.

3. The viable bacteria of non-treated specimens after light exposure shall be more than 1×10^3 cells for all three specimens.

4. After being kept in a dark place the viable bacteria of non treated specimens shall be more than 1×10^3 cells for all three specimens.

Photocatalyst antibacterial activity value calculation

$R_L = [\log(B_L / A) - \log (C_L/A)] = \log[B_L/C_L]$

R_L is the photocatalyst antibacterial activity value after light exposure

A is the average number of viable bacteria of non-treated samples just after inoculation

B_L is the average number of viable bacteria of non treated specimens after light exposure

C_L is the average number of viable bacteria of photocatalytic treated specimens after light exposure

$\Delta R = \log[B_L/C_L] - \log[B_D/C_D]$

ΔR is the photocatalyst antibacterial activity value with UV irradiation

B_D is the average number of viable bacteria of non – treated specimens after being kept in a dark place

C_D is the average number of viable bacteria of photocatalytic treated specimens after being kept in a dark place

Glass adhesion method

$M = P \times 20$

M is the number of cells of viable bacteria

P is the bacteria concentration (cells/ml)

20 is the quantity of PBS used for washout (ml)

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Propagation values for validation of conditions for a valid test

$F_{BL} = M_{BL} - M_{BA}$

F_{BL} is the growth value after light exposure

M_{BL} is the average logarithmic value of the number of bacteria for 3 non treated specimens after light exposure

M_{BA} is the average logarithmic value of the number of viable bacteria for three non treated specimens just after inoculation

$F_{BD} = M_{BD} - M_{BA}$

F_{BD} is the growth value after being kept in a dark place

M_{BD} is the average logarithmic value of the number of viable bacteria for three non treated specimens after being kept in a dark place

Photocatalyst antibacterial activity value calculation

$S_L = M_{BL} - M_L$

S_L is the photocatalyst antibacterial activity value after light exposure

M_L is the average logarithmic value of the number of viable bacteria for 3 photocatalytic treated specimens after light exposure

$\Delta S = (M_{BL} - M_L) - (M_{BD} - M_D)$

ΔS is the photocatalyst antibacterial value with light exposure

M_D is the average logarithmic value of the number of viable bacteria for three photocatalytic treated specimens after being kept in a dark place

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Results

1. Stainless steel

Sample description	Dilution	Colony count	Number of viable bacteria recovered per specimen	Log values
Untreated 1 t = 0	1 x 10^{-1}	TNTC	327500	5.5152113
	1 x 10^{-2}	64, 67		
	1 x 10^{-3}	6,7		
Untreated 2 t=0	1 x 10^{-1}	TNTC	362500	5.55930801
	1 x 10^{-2}	73, 72		
	1 x 10^{-3}	7, 8		
Untreated 3 t=0	1 x 10^{-1}	TNTC	370000	5.56820172
	1 x 10^{-2}	70, 78		
	1 x 10^{-3}	6, 7		
Light untreated 1	1 x 10^{0}	TNTC	17250	4.2367891
	1 x 10^{-1}	35, 34		
	1 x 10^{-2}	3, 4		
Light untreated 2	1 x 10^{0}	TNTC	21250	4.32735893
	1 x 10^{-1}	30, 55		
	1 x 10^{-2}	3, 5		
Light untreated 3	1 x 10^{0}	TNTC	11000	4.04139269
	1 x 10^{-1}	20, 24		
	1 x 10^{-2}	2, 1		
Light treated 1	1 x 10^{0}	12, 5	425	2.62838893
	1 x 10^{-1}	1, 2		
	1 x 10^{-2}	0, 0		
Light treated 2	1 x 10^{0}	5, 6	275	2.43933269
	1 x 10^{-1}	1, 0		
	1 x 10^{-2}	0,0		

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Sample description	Dilution	Colony count	Number of viable bacteria recovered per specimen	Log values
Light treated 3	1×10^{0}	11, 29	1000	3
	1×10^{-1}	1, 2		
	1×10^{-2}	0, 1		
Dark untreated 1	1×10^{0}	TNTC	49750	4.69679309
	1×10^{-1}	95, 104		
	1×10^{-2}	9, 10		
Dark untreated 2	1×10^{0}	TNTC	41500	4.6180481
	1×10^{-1}	77, 89		
	1×10^{-2}	6, 12		
Dark untreated 3	1×10^{0}	TNTC	41750	4.62065648
	1×10^{-1}	73, 94		
	1×10^{-2}	9, 10		
Dark treated 1	1×10^{0}	TNTC	25553	4.40744189
	1×10^{-1}	53, 51		
	1×10^{-2}	5, 8		
Dark treated 2	1×10^{0}	TNTC	14000	4.14612804
	1×10^{-1}	33, 23		
	1×10^{-2}	0, 1		
Dark treated 3	1×10^{0}	TNTC	25250	4.40226138
	1×10^{-1}	50, 51		
	1×10^{-2}	5, 10		

Test requirement fulfilment validation

1. 5.55930801-5.5152113/5.5473333 = 0.0079

Requirement is fulfilled

2. Logarithmic value of bacteria after inoculation must be within 1×10^{5} to 4×10^{5} range

Requirement is fulfilled

3. The viable bacteria in non-treated specimens following light exposure is greater than 1×10^{3} cells for all three specimens

Requirement is fulfilled

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4. The viability of bacteria from non-treated specimens after being kept in a dark place is greater than 1 x 10^3 cells for all three specimens

Requirement is fulfilled

Photocatalyst antibacterial activity value calculation

R_L = log[16500/566]= 29.15
=1.464

ΔR = log[16500/566] – log[44333/21601]
= log[29.15] – log[2.05]
= 1.464 – 0.311
= 1.153

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2. Plastic

Sample description	Dilution	Colony count	Number of viable bacteria recovered per specimen	Log values
Untreated 1 t = 0	1×10^{-1}	TNTC	217500	5.33745926
	1×10^{-2}	46, 41		
	1×10^{-3}	5, 11		
Untreated 2 t=0	1×10^{-1}	TNTC	197500	5.2955671
	1×10^{-2}	45, 34		
	1×10^{-3}	6, 2		
Untreated 3 t=0	1×10^{-1}	TNTC	210000	5.32221929
	1×10^{-2}	42, 42		
	1×10^{-3}	5, 3		
Light untreated 1	1×10^{0}	TNTC	47000	4.67209786
	1×10^{-1}	102, 86		
	1×10^{-2}	16, 15		
Light untreated 2	1×10^{0}	TNTC	42500	4.62838893
	1×10^{-1}	79, 91		
	1×10^{-2}	4, 5		
Light untreated 3	1×10^{0}	TNTC	17750	4.24919836
	1×10^{-1}	37, 34		
	1×10^{-2}	2, 5		
Light treated 1	1×10^{0}	54, 34	2200	3.34242268
	1×10^{-1}	5, 3		
	1×10^{-2}	0, 0		
Light treated 2	1×10^{0}	73, 88	4025	3.60476588
	1×10^{-1}	7, 3		
	1×10^{-2}	0, 1		
Light treated 3	1×10^{0}	51, 58	2725	3.43536651
	1×10^{-1}	7, 9		
	1×10^{-2}	0, 1		

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Sample description	Dilution	Colony count	Number of viable bacteria recovered per specimen	Log values
Dark untreated 1	1×10^{0}	TNTC	34000	4.53147892
	1×10^{-1}	64, 72		
	1×10^{-2}	5, 6		
Dark untreated 2	1×10^{0}	TNTC	40000	4.60205999
	1×10^{-1}	79, 81		
	1×10^{-2}	7, 9		
Dark untreated 3	1×10^{0}	TNTC	40500	4.60745502
	1×10^{-1}	77, 85		
	1×10^{-2}	9, 10		
Dark treated 1	1×10^{0}	TNTC	19250	4.28443073
	1×10^{-1}	40, 37		
	1×10^{-2}	4, 6		
Dark treated 2	1×10^{0}	TNTC	22250	4.34733002
	1×10^{-1}	53, 36		
	1×10^{-2}	3, 7		
Dark treated 3	1×10^{0}	TNTC	19750	4.2955671
	1×10^{-1}	30, 49		
	1×10^{-2}	3, 5		

Test requirement fulfilment validation

1. 5.337-5.29/5.318 = 0.008
Requirement is fulfilled

2. Logarithmic value of bacteria after inoculation must be within 1×10^{5} and 4×10^{5} range
Requirement is fulfilled

3. The viable bacteria in non-treated specimens following light exposure is greater than 1×10^{3} cells for all three specimens
Requirement is fulfilled

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4. The viability of bacteria from non-treated specimens after being kept in a dark place is greater than 1 x 10^3 cells for all three specimens

Requirement is fulfilled

Photocatalyst antibacterial activity value calculation

R_L = log[35750/2983]=
= log[12.23]
= 1.087

ΔR = log[35750/2983] – log[38166/20416]
= log[12.23] – log[1.86]
= 1.087 – 0.27
= 0.817

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3. Textile

Sample description	Dilution	Colony count	Number of viable bacteria recovered per specimen	Log values
Untreated 1 t = 0	1×10^{0}	132, 198	6600	3.81954394
	1×10^{-1}	4, 15		
Untreated 2 t=0	1×10^{0}	216, 284	10000	4
	1×10^{-1}	24, 21		
Untreated 3 t =0	1×10^{0}	183, 125	6160	3.78958071
	1×10^{-1}	14, 23		
Light untreated 1	1×10^{0}	252, 260	10240	4.01029996
	1×10^{-1}	25, 32		
Light untreated 2	1×10^{0}	247, 267	10280	4.01199311
	1×10^{-1}	30, 21		
Light untreated 3	1×10^{0}	300, 282	11640	4.06595298
	1×10^{-1}	30, 34		
Light treated 1	1×10^{0}	23, 27	1000	3
	1×10^{-1}	2, 2		
Light treated 2	1×10^{0}	6, 11	340	2.53147892
	1×10^{-1}	2, 0		
Light treated 3	1×10^{0}	6, 5	220	2.34242268
	1×10^{-1}	1, 0		
Dark untreated 1	1×10^{0}	TNTC	26000	4.41497335
	1×10^{-1}	73, 57		
Dark untreated 2	1×10^{0}	TNTC	34400	4.53655844
	1×10^{-1}	78, 94		
Dark untreated 3	1×10^{0}	TNTC	28800	4.45939249
	1×10^{-1}	65, 79		
Dark treated 1	1×10^{0}	179, 172	7020	3.84633711
	1×10^{-1}	13, 14		

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Sample description	Dilution	Colony count	Number of viable bacteria recovered per specimen	Log values
Dark treated 2	1×10^{0}	163, 182	6900	3.83884909
	1×10^{-1}	13, 19		
Dark treated 3	1×10^{0}	212, 227	8780	3.94349452
	1×10^{-1}	14, 20		

Test requirement fulfilment validation

1. F_{BL} = 4.028 – 3.869 = 0.159
F_{BL} is greater than 0 therefore parameter is validated

2. F_{BD} = 4.469 – 3.869 = 0.6
F_{BD} is greater than 0 therefore parameter is validated

S_L = 4.028 – 2.62 = 1.408
ΔS = (4.028 – 2.62) – (4.469 – 3.87)
= 1.408 – 0.599
=0.809

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Discussion and Conclusion

In accordance with the wishes of the client, the ISO 22447:2009 protocol was modified slightly as the surfaces to be tested were stainless steel, hard plastic and textile samples.

In understanding the data it must be noted that R_L and S_L values account for the reduction of viability caused by the exposure of the treated surfaces to light on hard surfaces and textiles respectively. In contrast, ΔR and ΔS values address the reduction of bacterial viability caused by the coating becoming light activated while accounting for the reduction in viability caused by the same coatings in a dark environment. From the data presented here it is clear that the coatings resulted in a reduction in bacterial viability even when stored in a dark place. It is therefore evident that the coatings in the absence of light have a bactericidal effect against *E. coli*, with photocatalyst antibacterial activity values of 1.153, 0.817 and 0.809 on stainless steel, clear plastic and textile respectively with all conditions for a valid test satisfied.

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*** End of Report***

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