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TEST REPORT

Al Maha Ceramics SAOG
PO Box 482,postal code 322
Falaj Al Qabail, Sohar, Sultanate of Oman

Report No: WAM18-0707/01
Sample No: WAM18-0707/01
Report Date: 11/10/2018

Sample description	: Al Maha Ceramics Glazed tiles: ABI -0.5
Sample Date Received	: 25/09/2018
Date of Test	: 25/09/2018 - 08/10/2018
Tested By	: PS

1.0 Introduction:

Further to the requisition received from **Al Maha Ceramics SAOG** dated 25th September 2018, the sample of Ceramic Tiles was tested for Antibacterial Activity.

2.0 Test method reference:

IHP in combination with ISO 22196:2007 and BS ISO 27447:2009; Fine ceramics (advanced ceramics, advanced technical ceramics) - Test method for antibacterial activity of semiconducting photo catalytic materials.

3.0 Principle:

This method is used to obtain the antibacterial photo catalytic materials by contact of a specimen with bacteria, under UV Light radiation. The film adhesion method is available for flat sheet, board or plate shaped materials. The specimen is laid in a Petri dish and the bacterial suspension is dripped onto the specimen. Then the adhesive film is placed on the suspension and the moisture conservation glass is placed on top of the Petri dish. The Petri dish containing the specimen is exposed to light. After exposure, the test bacteria are washed out of the specimen and the adhesive film. This washout suspension is measured by the viable bacterial count method.

4.0 Material and Reagents:

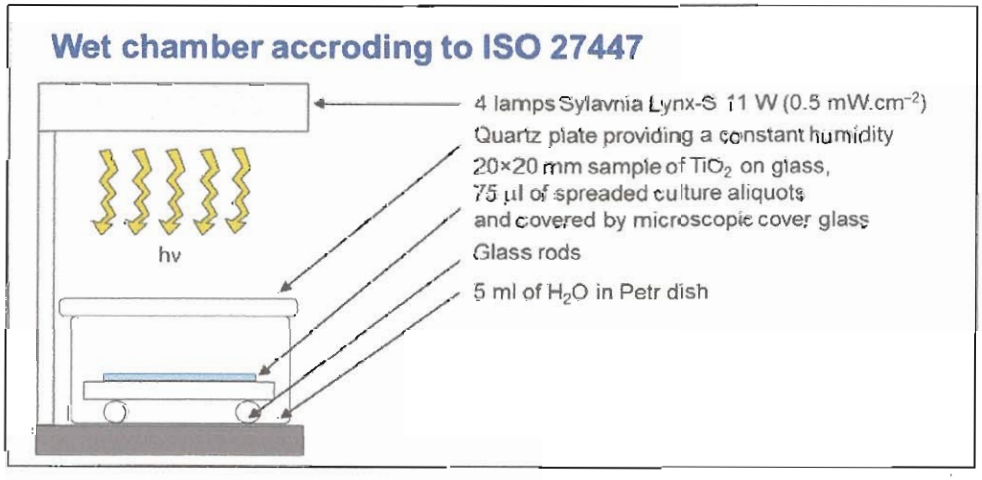
1) Bacterial Strain- *Staphylococcus aureus, Escherichia coli*
2) Nutrient Broth
3) Nutrient Agar
4) SCLDP – soybean Casein Digest Broth with Lecthin and Polysorbate 80
5) Physiological saline solution

6) Physiological saline solution for washout
7) Non-ionic surfactant- Polyoxyethylene sorbitan monooleate (Polysorbate 80)
8) An adhesive film with transparency rate of 85% for 340nm to 380nm or An adhesive glass which is a glass pane with a thickness less than or equal to 1.1mm with a transparency of rate over 85% for 340nm to 380 nm range.
9) Moisture preservation glass with transparency rate of 85% for 340nm to 380nm.
10) Glass Rod in a U shape which fits the petri plate
11) Glass Slide of dimension 50mm by 50mm
12) Black Light fluorescent lamp.

5.0 Method:

5.1 Film Adhesion Method

5.1.1 Lay a sterilized moisture control paper filter in the bottom of a sterilized Petri dish, add an adequate quantity of sterilized water, intercalate a glass tube or glass rod in order to avoid contact between the test piece and the paper filter, and place the test piece on it with the indoor light-active photocatalyst treated surface up.



5.1.2 Collect exactly 0.15 ml test bacterial suspension with a sterilized pipette and drip it onto each test piece. Put a film on top of the dripped suspension and lightly push to get the suspension spread to the whole film surface, while taking care that no suspension leaks out of the film edge. Then place a moisture conservation glass on the top of Petri dish. Except for 3 non-treated specimens for viable cell count performed just after test bacterial suspension is inoculated, proceed with illumination test.

5.1.3 Expose to light the Petri dishes containing the specimens (3 non-treated specimens and 3 indoor light active photocatalytic treated specimens) with bacterial suspension for 8 hour. For the 3 non-treated bacterial suspensions inoculated specimens for the test (post-inoculation specimen of test bacteria), put the adherence film and non-treated test piece in a Stomacher bag using sterilized tweezers, taking care to bacterial suspension leakage from film and non-treated test piece.

5.1.4 Add 10 ml of SCDLP, rub the specimens and the film well from outside the Stomacher bag by hands and washout the test bacteria. Quickly bring this washout solution to perform measurement of number of viable cells. Alternative equivalents of Stomacher bag may be used if they can be shown to lead to the same results. Also keep one set of the specimens in Dark room for analysis and one set for control analysis.

5.2 Measurement of number of living bacteria

5.2.1 1 ml of washout solution is taken with a sterilized pipette and added to (9 ± 0,1) ml of physiological saline solution in a test tube and thoroughly agitated. 1 ml of the solution is extracted with a new sterilized pipette and added to another test tube containing (9 ± 0,1) ml of physiological saline solution and thoroughly agitated again.

5.2.2 This process is repeated to obtain a series of dilutions, in compliance with the 10- times dilution method. 1 ml of the solution from the tubes of each series is extracted with new sterilized pipettes and placed in two Petri dishes each. 15 ml to 20 ml of nutrient agar kept at 45 °C to 48 °C is added in each Petri dish, allow them to stand for 15 minutes at room temperature.

5.2.3 When the agar medium solidifies, the Petri dishes are placed upside down and incubated for 40 h to 48 h at (37 ± 1) °C. Colony numbers are counted in the series Petri dishes with 30 colonies to 300 colonies.

5.2.4 The bacterial concentration of washout liquid is obtained by Equation (1) at two significant digits.

$P = Z \times R;$

Where P is the bacteria concentration (cells/ml); ---------- (1)

Z is the average number of colonies in 2 Petri dishes; DF is the dilution factor;

R is the dilution factor

6.0 Result:

7.1 Size of the Sample: The provided tile sample was cut to a dimension of 50±2 mm x 50±2 mm; with a thickness of 10 mm

7.2 Size of the Glass Pane (Non- Treated Specimen) : The Glass pane used as a Non Treated Specimen was cut to a dimension of 50±2 mm x 50±2 mm; with a thickness of 10 mm

7.3 Bacteria Used

The bacterial strains used for the Experiment were:-

i. *Staphylococcus aureus*

ii. *Escherichia coli*

7.4 Intensity of the UV irradiation: 0.25mW/cm^2 was the UV intensity

7.5 Light exposure Duration: 8 hours

7.6 Test Result for *Staphylococcus aureus*

Incubation conditions	Sample	CfU/sample	RL	ΔR
Control	Uncoated	600000	2.8	4.7
U.V (0.25 mW/cm^2)	Uncoated	50000		
	Coated	100		
Dark	Uncoated	600000		
	Coated	3600		

Table -1

7.7. Test Result for *Escherichia coli*

Table -2

Incubation conditions	Sample	CfU/sample	RL	ΔR
Control	Uncoated	2500000	2.4	4.2
U.V (0.25 mW/cm^2)	Uncoated	70000		
	Coated	250		
Dark	Uncoated	2500000		
	Coated	4000		

Where:-

RL= the photocatalyst antibacterial activity value after the UV irradiation intensity L

ΔR= the photocatalyst antibacterial activity value with the UV irradiation.

Sl. No.	Strains	Percentage Reduction	R-Value
1.	*Staphylococcus aureus*	99.98%	> 3
2.	*Escherichia coli*	99.99%	> 3

8.0 Conclusion

A pass or fail criterion is not mentioned in the current standard, so we uses the following criterion to comment on the level of activity determined.

Antibacterial activity	% kill	Comment
<1.5	<96.8	Poor
1.5- 2.0	96.9-99.0	Borderline
2.0-3.0	>99.0-99.9	Good
>3.0	>99.98	Excellent

Therefore it can be concluded from the study that the sample has excellent antibacterial activity against the tested strains with **Al Maha Ceramics Glazed tiles: ABI -0.5**

Signed for and on behalf of Wimpey Laboratories

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Test result relate only to the samples tested.
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