Campden Technology Limited
Station Road
Chipping Campden
Gloucestershire
GL55 6LD, UK

Tel: +44 (0)1386 842000
Fax: +44 (0)1386 842100
www.campden.co.uk

ASSESSMENT OF ANTIMICROBIAL PROPERTIES OF SiO_2 COATINGS ACCORDING TO THE PRINCIPLES OF ASTM E2180

PROJECT: MB/REP/125353/2

REPORT AUTHORISED BY:

Dr. Gail Betts, Manager of Microbiological Safety and Spoilage Section

26th January 2012

1. BACKGROUND

The Client approached Campden with a request to test the antimicrobial efficacy of SiO_2 coatings.

Two liquid sprays were supplied: NX BF 2065 (antimicrobial) and NX 1051.

These were used to coat stainless steel discs (diameter 2cm) according to a procedure agreed with the client. The agreed procedure was to:

1) Spray NX BF 2065 or NX 1051 onto the surface of stainless steel
2) Wipe across surface with a cloth to ensure even coverage
3) Dry for 10 minutes
4) Buff with a clean cloth
5) Heat for 10 minutes to harden the surface either in a hot oven or using a hair dryer

The non-coated material was sprayed with sterile distilled water and the above process repeated.

The antimicrobial properties were assessed according to the protocols described in ASTM E2180. In summary, the procedure consisted of applying an agar slurry containing test organisms onto the surface of treated material and control material. The samples were incubated for 24h and the levels present on the treated materials and coated materials at the end of this time were used to calculate the efficacy of the coatings. This procedure is described in more detail below.

2. EXPERIMENTAL PROTOCOL AND METHODS

2.1 Products

Two products were supplied in small spray bottles. These were NX BF 2065 (antimicrobial) and NX 1051. These were delivered on the 14/11/11 and testing commenced on 17/11/11.

Subsequent correspondence from the manufacturer of the coatings has indicated that the bottles received at Campden BRI had been labelled the wrong way round prior to the bottles being received by the client, i.e. the bottle labelled NX BF 2065 actually contained the coating NX 1051 and the bottle labelled NX 1051 actually contained the coating NX BF 2065. Based on this information, the codings for the two coatings have been transferred to the correct bottles.

These compounds could be used to coat the surface of a range of materials. In this instance, stainless steel discs were used.

The coatings were applied to the discs using a protocol defined by the client as described above. Three discs were needed for each coating and for each of three organisms at time 0 and time 24h. Thus a total of 54 discs were required, 18 for each coating and 18 for the control.

2.2 Culture preparation

Three different organisms were used for this trial, *Salmonella*, *Staphylococcus aureus* and *Escherichia coli*.

Three strains of each organism were used as shown below. Each organism was grown separately in Nutrient Broth (NB, Oxoid CM0001) for 18-24h at 37 ± 1ºC.

The three *Salmonella* strains were added together to make a single inoculum containing all three strains. The same was done for the *Staph. aureus* and *E. coli* strains.

The strains were used were:

Salmonella Typhimurium CRA 1962
Salmonella Typhimurium CRA 1960
Salmonella Enteritidis CRA 1868
E. coli CRA 11626
E. coli CRA 11017
E. coli CRA 16559
Staph. aureus 4105
Staph. aureus 7138
Staph. aureus 1244

2.3 Inoculation of discs

An agar slurry was made using 0.85g NaCl and 0.3g agar dissolved in 100ml deionised water and sterilised at 121ºC/15minutes.

Separate portions of the agar were used for each of the three organisms. A sample (1ml) of each microbial suspension containing approximately $1x10^8$ cells per ml was added to the agar slurry to give a final level of approximately $1x10^6$ /ml of agar.

0.5ml volumes of the inoculated slurry was placed onto the surface of each stainless steel disc and allowed to solidify.

Three samples of each surface/organism combination were evaluated at time 0 and a further three were evaluated after 24 ± 2h incubation at 30 ± 1ºC.

2.4 Microbiological testing

Each disc was added to a sterile pot containing 8ml neutralising agent (British Standard Inactivator) and sterile glass beads. The pots were shaken well and vortex mixed to ensure the agar slurry was removed from the surface of the discs.

The samples were diluted in Maximum Recovery Diluent (MRD, LabM LAB 103) and the levels of organisms present were enumerated using the pour plate technique with Plate Count Agar (PCA, LabM LAB149), incubated at 30 ± 1ºC for 48 ± 4h. All resultant colonies were counted and calculated as cfu/ml.

3 RESULTS

The results from the trials are shown in Tables 1, 2 and 3 for the three organisms. The levels of organisms present at time 0 and time 24 are given in the Table. The time 0 counts are not used in the final calculation but serve as a control to ensure that the cultures were viable and at the correct concentration at the start of the trial.

The efficacy of the compounds is calculated using an equation given in ASTM E2180 where the level of organisms present on the treated samples at time 24 is compared to the level of organisms present on the control samples after time 24 using the following equation:

$$\% \text{ reduction} = [(a-b) \times 100] / a$$

a = the antilog of the geometric mean of organisms recovered from the incubation period control samples

b = the antilog of the geometric mean of the number of organisms recovered from the incubation period treated samples

The level of reduction present after the 24h test are shown in Table 4.

Table 1: Levels of *Salmonella* present on stainless steel discs

Product	Rep	0h cfu/g	24h cfu/g
NX BF 2065	a	6.46E+05	1.19E+06
NX BF 2065	b	6.97E+05	5.03E+03
NX BF 2065	c	7.99E+05	1.38E+05
	Mean	**7.14E+05**	**4.44E+05**
NX 1051	a	5.61E+05	4.08E+08
NX 1051	b	1.00E+06	1.02E+08
NX 1051	c	7.99E+05	1.96E+03
	Mean	**7.88E+05**	**1.70E+08**
Control	a	4.93E+05	1.70E+07
Control	b	8.33E+05	1.02E+08
Control	c	8.16E+05	3.40E+08
	Mean	**7.14E+05**	**1.53E+08**

Table 2: Levels of *Staph. aureus* present on stainless steel discs

Product	Rep	0h cfu/g	24h cfu/g
NX BF 2065	a	2.89E+06	1.00E+00
NX BF 2065	b	1.87E+06	3.23E+06
NX BF 2065	c	1.70E+06	1.00E+00
	Mean	**2.15E+06**	**1.08E+06**
NX 1051	a	1.58E+06	8.16E+04
NX 1051	b	3.06E+06	3.64E+03
NX 1051	c	2.38E+06	9.69E+02
	Mean	**2.34E+06**	**2.87E+04**
Control	a	3.06E+06	6.80E+06
Control	b	3.23E+06	4.59E+06
Control	c	3.23E+06	6.63E+06
	Mean	**3.17E+06**	**6.01E+06**

Table 3: Levels of *E. coli* present on stainless steel discs

Product	Rep	0h cfu/g	24h cfu/g
NX BF 2065	a	1.56E+05	5.27E+07
NX BF 2065	b	2.55E+05	6.46E+07
NX BF 2065	c	5.10E+05	1.26E+02
	Mean	**3.07E+05**	**3.91E+07**
NX 1051	a	7.99E+05	1.00E+08
NX 1051	b	6.97E+05	1.46E+08
NX 1051	c	7.65E+05	1.48E+08
	Mean	**7.54E+05**	**1.31E+08**
Control	a	7.65E+05	4.59E+08
Control	b	6.80E+05	5.61E+08
Control	c	5.44E+05	5.61E+08
	Mean	**6.63E+05**	**5.27E+08**

Table 4:
Calculated % reduction in levels of organisms according to ASTM E2180

Organism	NX BF 2065	NX 1051
Salmonella	99.9%	94.8%
Staph. aureus	99.99%	99.9%
E. coli	99.9%	75.3%

4. CONCLUSIONS

The level of reduction observed in these studies ranged from 75% to 99.99%. In 4 of the 6 trials, the level of reduction was greater than 99%.

Coating NX BF 2065 was the most effective compound tested with >99.9% reduction for all three organisms.

Staph. aureus appeared to be the most susceptible organism to both compounds with 99.99% reduction after 24h for both compounds.

E. coli was the least susceptible to NX 1051 with only a 75% reduction achieved for this compound.

Based on the calculations given in ASTM E2180, Compound NX BF 2065 had good antimicrobial properties against *Salmonella*, *Staph. aureus* and *E. coli* when compared to non-treated controls over a 24h test period. Compound NX 1051 had good antimicrobial properties against *Staph. aureus,* but was less effective against *Salmonella* and *E. coli*.

5. REFERENCE

Standard Test Method for Determining the Activity of Incorporated Antimicrobial Agents in Polymeric or Hydrophobic Materials. ASTM E2180 - 07.