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INDUSTRIAL MICROBIOLOGICAL SERVICES LTD

STUDY REPORT: Determination of Growth of *Escherichia coli*, *Enterococcus hirae*,
Staphylococcus aureus and *Pseudomona aeruginosa* on the Surface of
Wall-Tech UV Coatings

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1 Introduction

This report describes the effect of Wall-Tech UV coatings from E Wood Ltd on bacterial growth / survival on its surface using a method under development as an ISO Standard by the International Biodeterioration Research Group (IBRG) and based on the Japanese Industrial Standard JIS Z 2801: 2000.

2 Test Materials

Panels (*ca* 200 x 300 mm) which had been coated with Wall-Tech UV prepared using the production facilities at E Wood Ltd were supplied. An uncoated, standard non-porous polyester sheet was employed as the control. All samples were held in the dark at 20°C prior to testing.

3 Methods

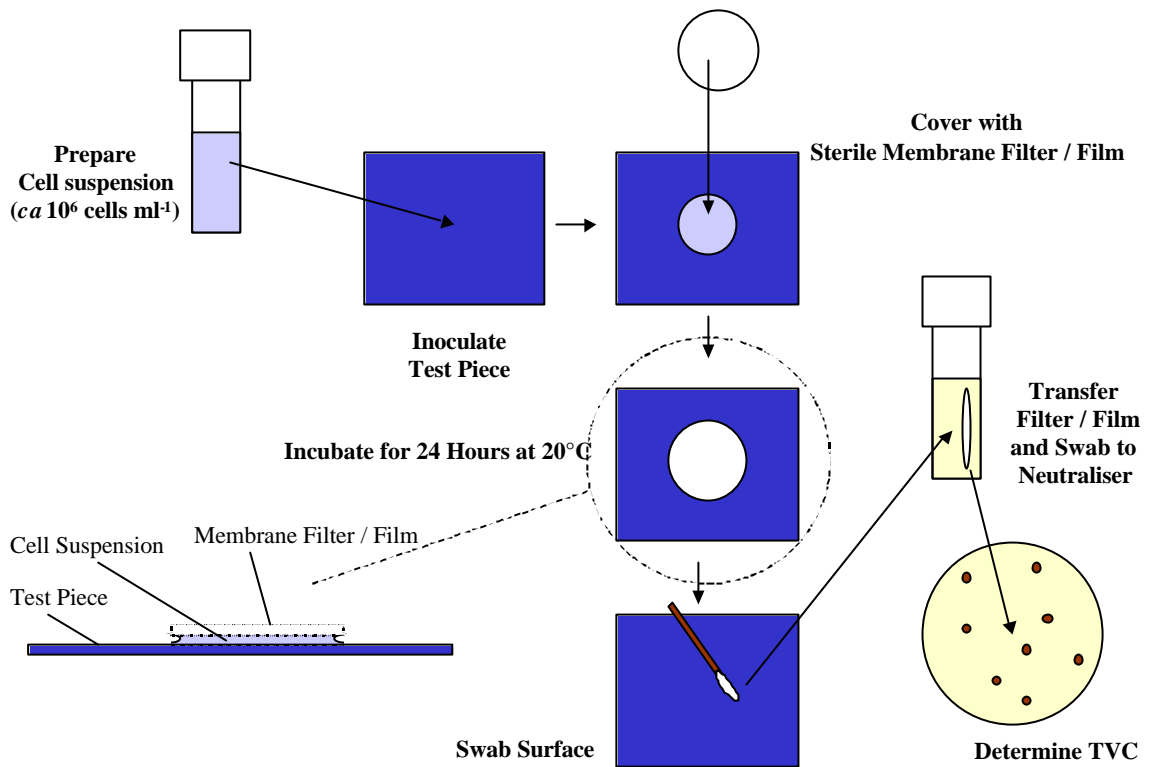
Bacterial growth / survival was determined by exposing suspensions of a range of species of bacteria to the test coating for up to 96 hours. The individual suspensions were held in intimate contact with the coated surface in the absence of nutrients but under isotonic conditions and their viability was measured at intervals by total viable count.

3.1 Bacterial Survival on Surfaces

To determine the survival of bacteria on the test surfaces, samples were analysed using a method developed from the Japanese Film Adherence Method (Ref. 1) by the IBRG.

Growth / survival on the test coating was determined using 4 species of bacteria individually. Twelve replicate aliquots (159µl) of a log phase cell suspension of *Escherichia coli*, (3.0×10^6 cells ml⁻¹: equivalent to 5.2×10^5 cells cm⁻²), *Enterococcus hirae* (3.8×10^6 cells ml⁻¹: equivalent to 6.8×10^5 cells cm⁻²), *Staphylococcus aureus*, (1.7×10^6 cells ml⁻¹: equivalent to 3.0×10^5 cells cm⁻²), *Pseudomonas aeruginosa* (1.9×10^6 cells ml⁻¹: equivalent to 3.4×10^5 cells cm⁻²) in ¼-strength Ringers' solution were placed on the surface of both the test coating and the polyester film control. The individual suspensions were held in intimate contact with an area of approximately 9 cm² using a polyethylene film for up to 96 hours at 20°C. After 0, 24, 48 and 96 hours contact, the populations on three replicate areas were recovered by swab and by transferring the cover into sterile distilled water. The size of the viable population associated with these areas was then enumerated by spiral dilution onto Trypcase Soya Agar. The method is described in schematic form in Figure 1 below.

Figure 1: Schematic of Surface Test Method



4 Results / Discussion

The results are shown in Tables 1 - 4 and Figure 2 - 5 below as Colony Forming Units (CFU) cm^{-2} .

It can be seen from the results below that no biologically significant growth of any the test bacterial species was observed on the test coating during this study. The viability profiles of all of the test strains followed the same pattern as observed on the blank non-porous polyester sheet and was typical of these strains under the conditions used except for *Ps aeruginosa*. It can be seen from the results below that the population of this organism lost viability rapidly when exposed to the test coating whereas the population exposed to the polyester sheet remained viable (and actually increased slightly in size). A similar trend was also observed with the *E coli*. Although there was a loss of viability on both the test surface and the control, a greater reduction was observed on the test surface after 96 hours incubation. However, this difference was not statistically significant ($P = 0.249$) whereas the differences observed with *Ps aeruginosa* were (see Table 4).

Table 1: Survival of *E coli* on Surface of Coating (as CFU cm⁻²)

Sample	Contact Time (Hours)			
	0	24	48	96
Control	5.2 x 10 ⁴	5.4 x 10 ⁴	5.8 x 10 ⁴	2.1 x 10 ⁴
Coating 1		5.9 x 10 ⁴	5.2 x 10 ⁴	8.9 x 10 ³
Significance Level (1 way ANOVA)		-	-	2.5 x 10 ⁻¹

Table 2: Survival of *Ent hirae* on Surface of Coating (as CFU cm⁻²)

Sample	Contact Time (Hours)			
	0	24	48	96
Control	6.7 x 10 ⁴	5.0 x 10 ⁴	6.8 x 10 ⁴	5.0 x 10 ⁴
Coating 1		6.5 x 10 ⁴	5.5 x 10 ⁴	3.9 x 10 ⁴

Table 3: Survival of *Staph aureus* on Surface of Coating (as CFU cm⁻²)

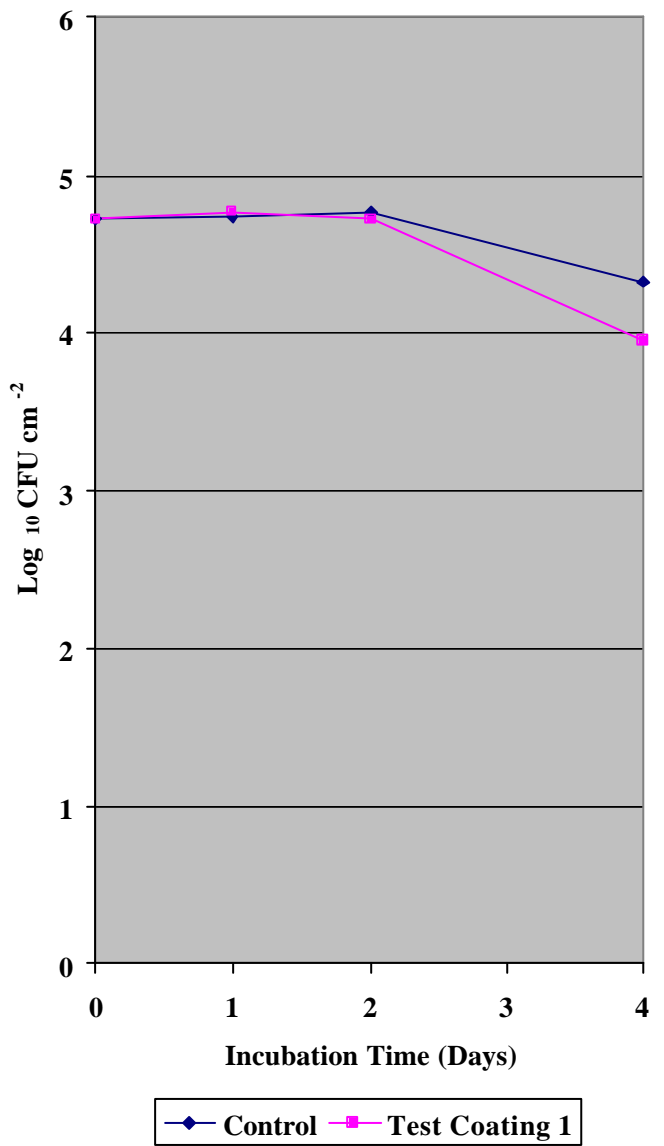
Sample	Contact Time (Hours)			
	0	24	48	96
Control	3.0 x 10 ⁴	3.0 x 10 ⁴	8.9 x 10 ⁴	1.7 x 10 ⁴
Coating 1		3.0 x 10 ⁴	6.8 x 10 ⁴	1.7 x 10 ⁴

Table 4: Survival of *Ps aeruginosa* on Surface of Coating (as CFU cm⁻²)

Sample	Contact Time (Hours)			
	0	24	48	96
Control	3.4 x 10 ⁴	3.6 x 10 ⁴	5.1 x 10 ⁴	5.0 x 10 ⁴
Coating 1		2.0 x 10 ⁴	1.9 x 10 ⁴	1.7 x 10 ¹
Significance Level (1 way ANOVA)		3.7 x 10 ⁻⁵	4.3 x 10 ⁻⁷	1.4 x 10 ⁻⁹

Figure 2: Determination of Bacterial Growth / Survival on Surface of Coating

Escherichia coli (ATCC 10536)



Enterococcus hirae (ATCC 8043)

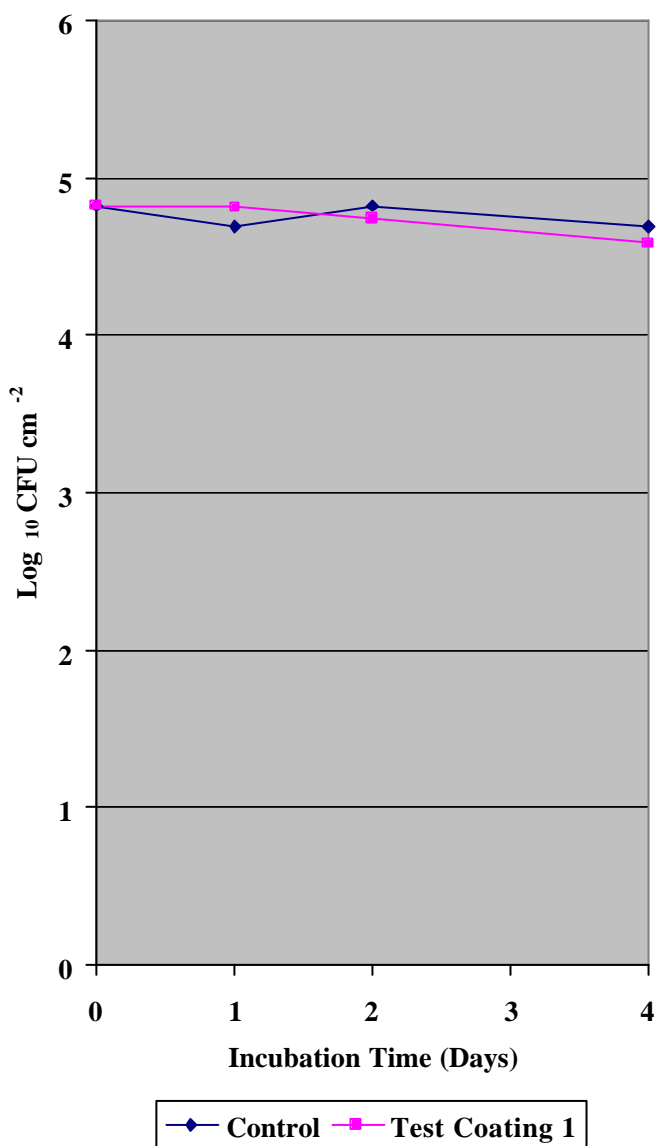
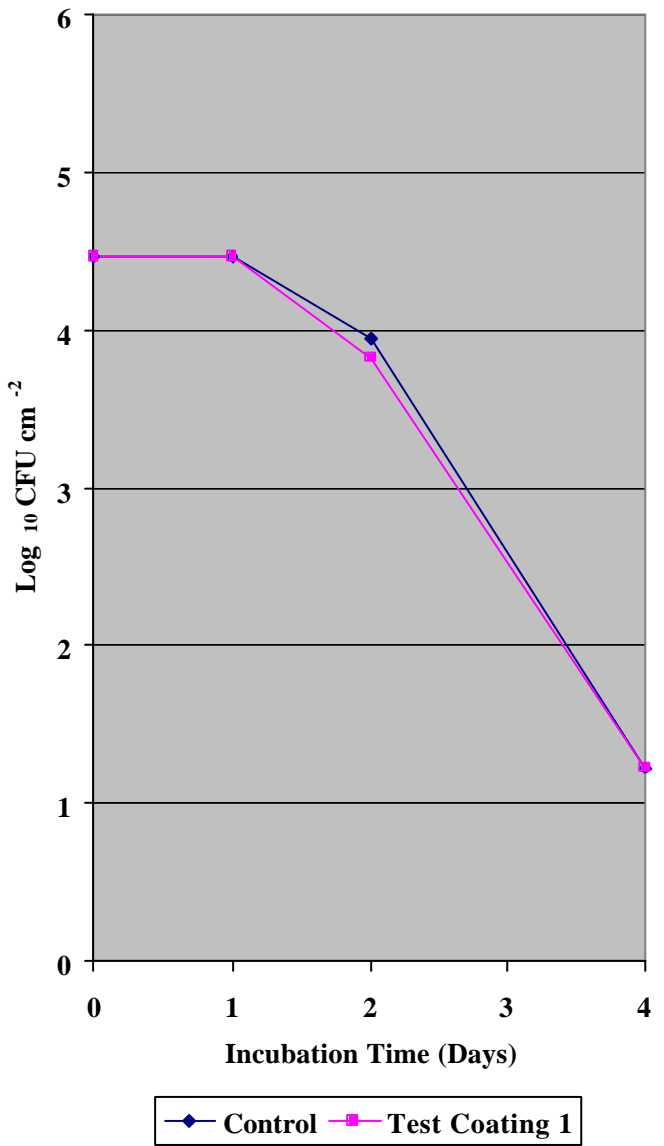
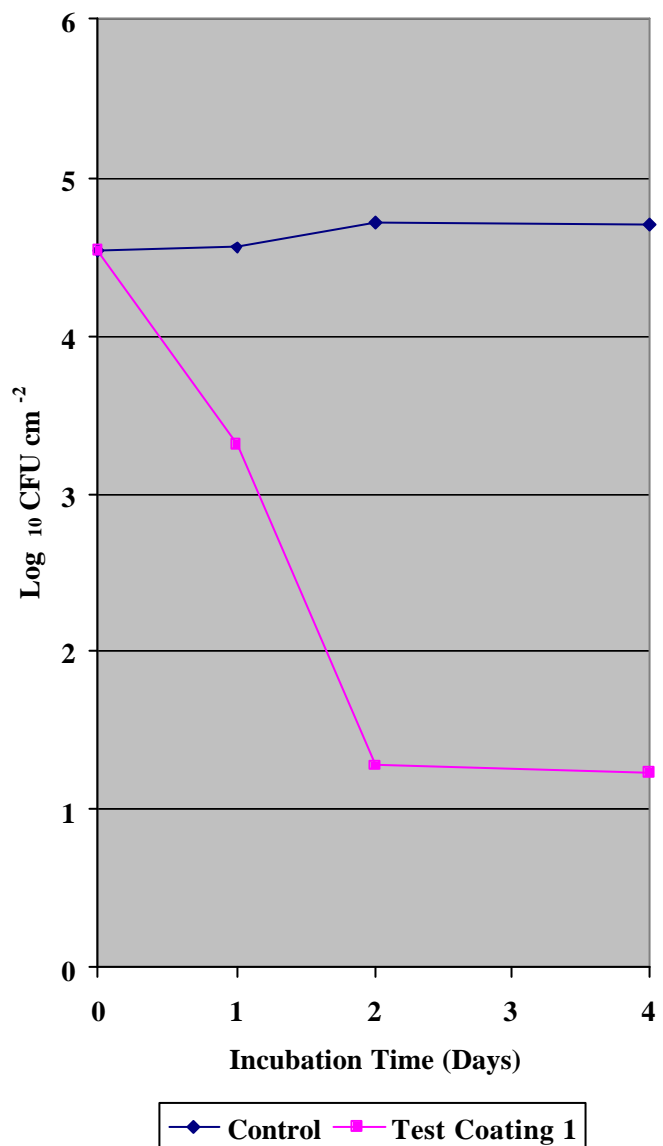


Figure 3: Determination of Bacterial Growth / Survival on Surface of Coating

Staphylococcus aureus (ATCC 6538)



Pseudomonas aeruginosa (ATCC 15442)



5 Raw Data

The raw data for this study will be held in file IMSL/2003/06/006 in the Archive of IMSL at Pale Lane, Hartley Wintney, Hants, RG27 8DH, UK for 6 years from the date of this report unless other specific instructions are given.

6 References

- 1 Japanese Industrial Standard JIS Z 2801: 2000 (E)

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