Effects of Surface Treatments on Formation and Removal of Biofilms: Some Preliminary Findings

Phanida Saikhwan
Department of Chemical Engineering, Faculty of Engineering, Thammasat Univeristy, Pathumthani 12121, Thailand

Abstract

Biofilms found at building exteriors are known to cause deterioration and degradation of the exteriors. One of the modern strategies to prevent biofilm formation is to apply coatings to surfaces. In this paper, preliminary investigations on the effects of surface properties on formation and cleaning of biofilms were illustrated. The surface properties investigated were surface energy (achieved by using different coating materials) and surface roughness (achieved by applying mechanical treatments). The biofilms studied were Pseudomonas fluorescens and Arthronema africanum. The results showed that surface roughness influences the formation of biofilms to a greater extent than surface energy. However, insignificant effects of both surface roughness and surface energy were observed on cleaning of biofilms.

* Corresponding author.
E-mail: psaikhwan@engr.tu.ac.th

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1. Introduction

Biofilms are broadly defined as assemblages of microorganisms and their associated extracellular products at an interface and typically attached to an abiotic or biotic surface (Davey & O'Toole, 2000). A biofilm can be formed by a single microorganism, but more often biofilms consist of many species of bacteria, as well as fungi, algae, protozoa, debris and corrosion products. Hence, biofilms are found in many forms, ranging from plaque on teeth to slimy layers on stones found at waterfalls.

Biofilms are also observed on building exteriors and are known to cause the deterioration and degradation of the exteriors, commonly known as "weathering" (Gaylarde & Gaylarde, 2005). Guillitte and Dreesen (1995) investigated the bioreceptivity of building materials and suggested that apart from environmental factors, surface roughness, initial porosity and the mineralogical nature of the substrate were key factors.

To control formation of biofilms or biofoulants, antifoulant paints are often used. These paints are simply toxic to microorganisms. However, the slimy layers found in biofilms can protect the microorganisms from the toxicity (Hellio et al., 2001). Moreover, the use of some toxic paints has been restricted, in particular for ship painting, as these paints pose risks to aquatic ecosystems (Tolosa et al., 1996). Several other attempts have been made to identify materials that do not promote or even suppress biofilm formation (Rogers et al., 1994; Klueh et al., 2000; Hashimoto, 2001). This includes incorporation of antimicrobial agents into surface materials or coatings (Meyer, 2003; Thouvenin et al., 2003). However, this method is currently limited to medical applications.

With recent developments in surface coating technology, various coatings have been used in fouling mitigation. The challenge then lies in the development and use of surface coatings, suitable to large-scale operations, to prevent biofilm formation and to ease cleaning (e.g. Chisholma et al., 2007; Dinua et al.,2007). Obviously, a better understanding of what surface properties prevent biofilm formation and ease cleaning is required. Hence, this paper showed some preliminary investigations on the effects of surface properties on the formation and cleaning of biofilms.

2. Material and Methods

The two biofilms studied were *Pseudomonas fluorescens* and *Arthronema africanum* to represent bacterial and algae biofilms respectively. The bacterial and algae biofilms were the major biomass found from biofilms collected from building exteriors (Gaylarde & Gaylarde, 2005). In order to clearly differentiate between the effects from surface energy (due to coatings) and roughness, stainless steel surfaces treated with various methods were used.

2.1 Microorganism and Culture

Pseudomonas fluorescens (ATCC 13525T) was used to produce biofilms. The nutrient solution for *P. fluorescens* was made from 5 g/l glucose, 2.5 g/l peptone and 1.25 g/l yeast extract, in 0.02 M phosphate buffer (KH_2PO_4: Na_2HPO_4). All reagents were analytical grade. Peptone, yeast extract and agar were purchased from Oxoid, UK; the salts for buffer and glucose were purchased from Acros Organics, UK. The nutrient solution for *Arthronema africanum* was made following the recipe given in Table 1. Deionised water was used in the experiment unless specified and was sterilized by autoclaving.

2.2 Biofilm Formation

2.2.1 Pseudomonas Fluorescens Biofilms

P. fluorescens biofilms were grown on stainless steels (SS316): uncoated, Diamond-like Carbon (DLC), SiCAN and SiCON® coated. The coated substrates were supplied by Fraunhöfer Institute for Surface Engineering and Thin Films, Braunschweig, Germany. Figure 1 illustrates the experimental set-up used for biofilm formation. The set-up and flow rates were modified by scaling down the set-up used by Pereira et al. (2002). A culture was grown at 27˚C and pH 7, in a 1 litre glass fermenter (F1). The temperature was controlled using a water bath whereas the pH was maintained by using nutrients containing buffer (0.02 M phosphate buffer). The fermenter (F1) was aerated (2 l/min) and continuously fed with a sterile nutrient solution (20 ml/h) using a peristaltic pump. The culture was used to continuously inoculate (20 ml/h) another 1 litre fermenter, which acted as a mixer (F2), initially containing sterile water. The mixer was aerated (1 l/min) and fed with sterile nutrient solution (5 ml/h) and sterile water (478 ml/h) to obtain a cell suspension with $6x10^7$ cells/ml (OD at 640 nm = 0.04) in a diluted nutrient (0.05 g/l glucose). The bacterial suspension was pumped through the fouling unit (2300 ml/min, *Re* ~ 1560) and back to the mixer (F2). The volume in the mixer was kept constant by an overflow drain. Both the fermenter and the mixer were suitably agitated (120 rpm), and kept at 27˚C using a water bath. The fouling unit shown in Figure 2 consists of two Perspex blocks each containing a duct of 15 mm square cross-section. The two blocks are separated by a sample plate so that the fouling unit operates as a simple co-current heat exchanger: the cell suspension is on the process side whereas water from the water bath, at 27˚C, is on the utility side. The conditions used to form the biofilm are summarised in Table 2.

(a) Food medium (800 ml)

$Na_2HPO_4.2H_2O$	208 mg
KH_2PO_4	592 mg
$CaCl_2$	8 mg
Fe.EDTA	8 mg
$MgSO_4.7H_2O$	40 mg
KNO_3	800 mg
Trace element mixture*	0.8 ml

(b) Trace element mixture (1 litre)

$Al_2(SO_4)_3.18H_2O$	3.58 g
$MnCl_2.4H_2O$	12.98 g
$CuSO_4.5H_2O$	1.83 g
$ZnSO_4.7H_2O$	3.20 g

Table 1. Recipe for making food medium for *Arthronema africanum.*

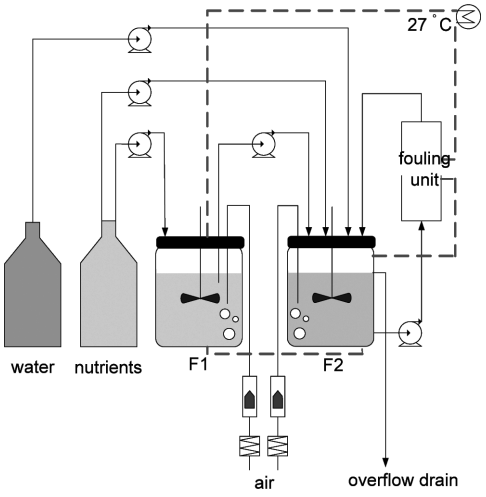

Figure 1. Schematic of biofilm formation system. F1 - Fermenter1, F2 – Mixer. Fouling Unit - see Figure 2. Dashed box represents where temperature was controlled.

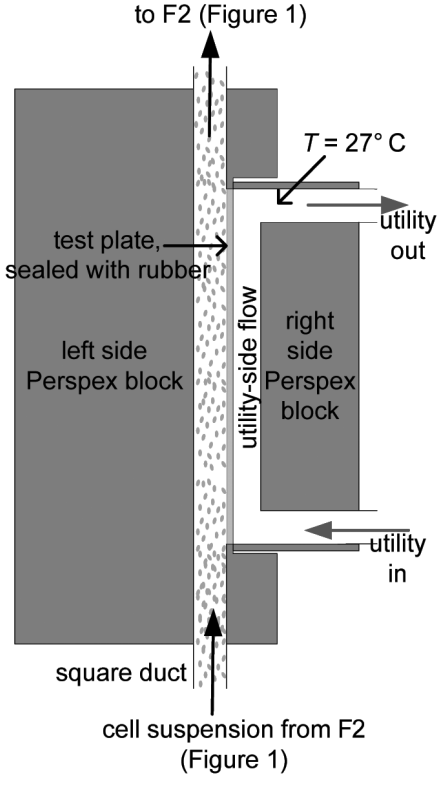

Figure 2. Schematic of biofilm fouling unit.

Table 2. Conditions for *P. fluorescens* biofilm formation.

Specific growth rate (F1)	0.02 per s
Dilution rate (F2)	~ 0.5 per s
Glucose concentration (F1)	5 g/l
Glucose concentration (F2)	0.05 g/l
Bulk temperature and surface temperature	27˚C
Re	1560
Mean velocity of flow, u_m	0.17 m/s

2.2.2 Arthronema Africanum Biofilms

Biofilm samples on stainless substrates of varying roughness were supplied by Katharina Bode, Technical University of Braunschweig (TU-BS), Germany. The biofilm had been grown from an inoculum of cyanobacteria called *Arthronema africanum* (SAG 1.89, OD at 640 nm = 0.04) in an air-lift reactor with a surface temperature (35˚C) slightly higher than that of the inoculum (25-30˚C). The reactor was aerated to saturation. The substrates were of varying roughness, namely untreated stainless steel (SS316), sandpaper roughened stainless steels grades K80 and K240, and electropolished stainless steel (supplied by Fraunhöfer Institute for Surface Engineering and Thin Films, Braunschweig, Germany).

2.3 Surface Characterization

The plates used as substrates for *P. fluorescens* biofilms were characterized by roughness measured by atomic force microscopy (AFM, Veeco Instrument, UK) and surface energy measured using a Drop Shape Analysis (DSA) device (DSA100, KRÜSS GmbH). The plates used as substrates for Arthronema africanum were rougher and their roughness was determined using a profilometer (PGK20, Mahr Instruments, Germany). The roughness of these substrates as well as the surface energy of all the sample plates were measured by T. Geddert, TU-BS, Germany.

2.4 Fluid Dynamic Gauging (FDG)

Fluid dynamic gauging (FDG) was used to measure the thickness of biofilms deposited on sample plates and the corresponding stress required to clean them (deposit strength measurements). Figure 3(a) depicts a schematic diagram of the FDG configuration: a gauging nozzle is fully submerged in liquid, and by applying a suction head (H) the liquid is withdrawn through a nozzle (diameter d_t) and a siphon tube (Figure 3(b)). The liquid flow rate, m, varies with the clearance between the nozzle and the surface of deposit, h, and hence deposit thickness (δ) can be measured as follows:

$$\delta = h - h_0 \qquad (1)$$

where h_0 is the clearance between the nozzle and the substrate when deposit is removed. Both h and h_0 can be measured using a micrometer connected to the FDG (M in Figure 3(a)).

Chew et al. (2004) showed that the shear stress imposed by the gauging flow, τ, under the nozzle rim, r, can be approximated using analytical expression described by Middleman (1998) and the maximum shear stress (τ_{max}) occurs under the inner radius of the rim i.e. at $r = \frac{d_t}{2}$. Hence, the maximum shear stress imposed on the deposit can be estimated as follows:

$$\tau_{max} = \frac{6m\mu}{\rho\pi h^2 d_t} \qquad (2)$$

where μ is the dynamic viscosity and ρ the density of liquid.

In this investigation, two sizes of nozzle were used (5 mm and 1 mm diameter) so that the stresses imposed on the biofilm would cover a wide range of values (shear stress range ~ 1.4 - 60 Pa).

3. Results and Discussion

Effect of surface coatings

The sample plates used in the system of *P. fluorescens* biofilms were similar in roughness (168-175 nm) and the surface energy values are reported in Table 3.

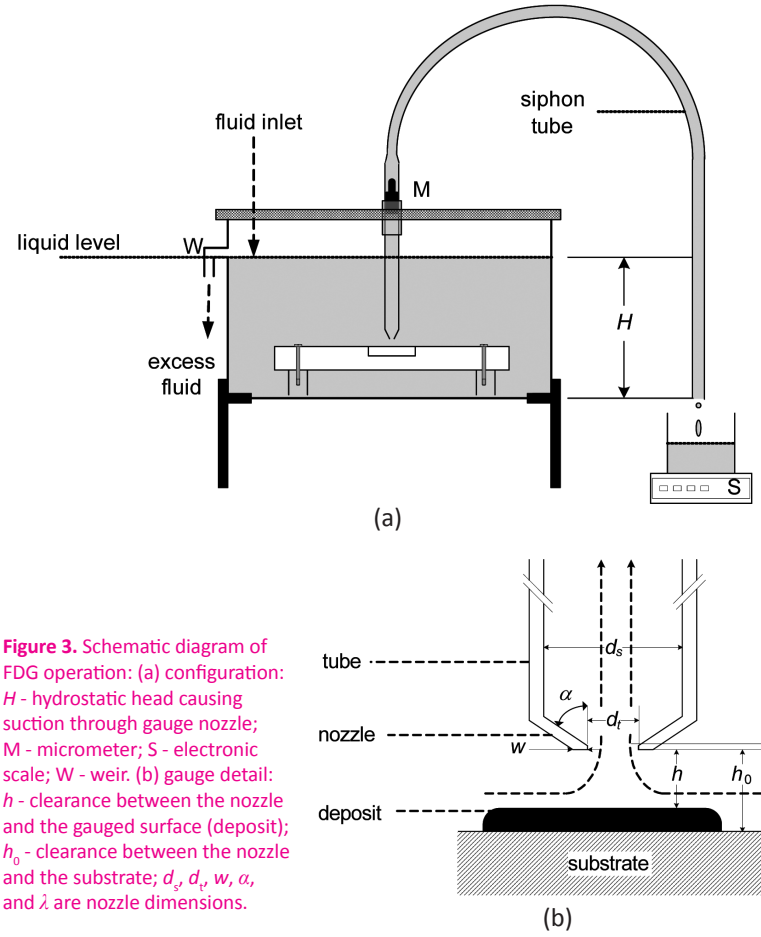

Figure 3. Schematic diagram of FDG operation: (a) configuration: H - hydrostatic head causing suction through gauge nozzle; M - micrometer; S - electronic scale; W - weir. (b) gauge detail: h - clearance between the nozzle and the gauged surface (deposit); h_0 - clearance between the nozzle and the substrate; d_s, d_t, w, α, and λ are nozzle dimensions.

Table 3. Surface energy values (Liftshitz Van der Waals) of sample plates for the system of *P. fluorescens* biofilms.

Sample plates	Surface energy (N/m)
Uncoated	39.98
SiCAN coated	35.65
SiCON® coated	32.07
DLC coated	45.80

P. fluorescens biofilms grown on coated stainless steels and uncoated stainless steel were similar in appearance and the similarity is also observed from AFM images (Figure 4). In addition, the thickness of these biofilms were similar (550-580 μm), as illustrated by the thickness at small shear stress (~1.5 Pa) in Figure 5. It should be noted that the thickness measurements using FDG were not shown for biofilms grown on SiCON® because the biofilm samples grown on SiCON® coated plates were destroyed in the initial adjustment of FDG. Nevertheless,

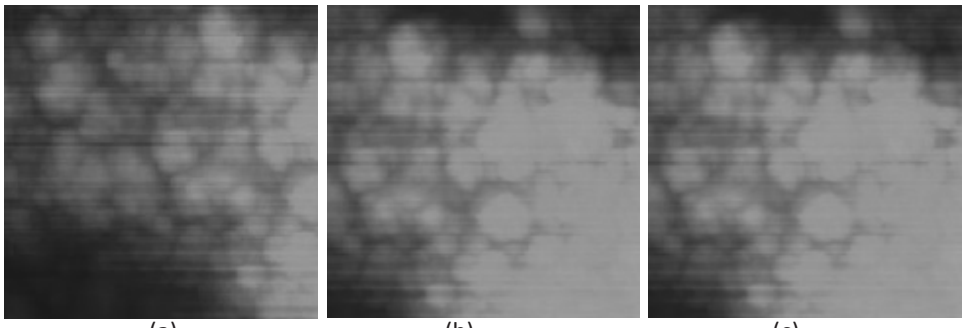

Figure 4. AFM images of *P. fluorescens* biofilms (2.2 x 2.2 µm, height scale 250 nm) deposited on (a) stainless steel; (b) SICON®; (c) DLC.

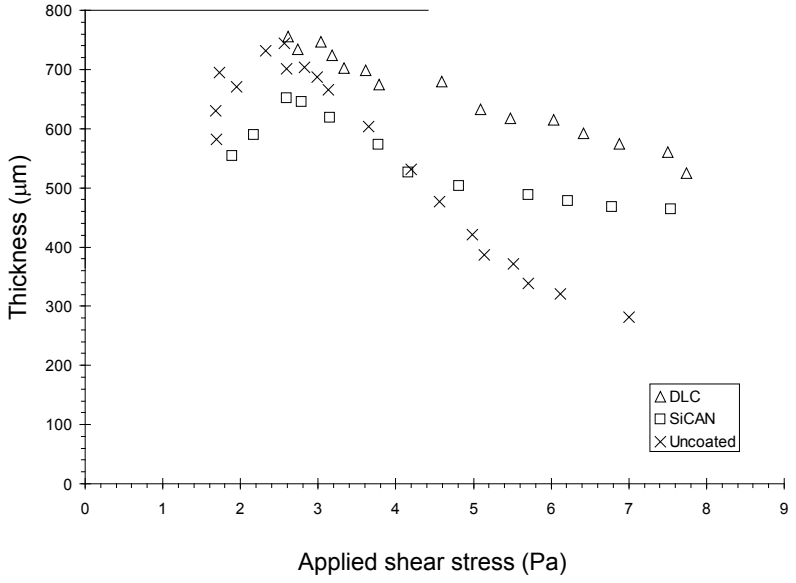

Figure 5. Thickness of *P. fluorescens* biofilms on coated and uncoated stainless steels vs. maximum applied shear stress.

Table 3. Surface energy values (Liftshitz Van der Waals) of sample plates for the system of *P. fluorescens* biofilms.

Sample plates	Ra (µm)
Untreated stainless steel	242
Sandpaper roughened (grade K80)	238
Sandpaper roughened (grade K240)	115
Electropolished	90

the preliminary result shows that surface energy (or surface coating materials) seems to have little influence on biofilm formation.

Figure 5 shows that at small shear stress, the response to shear stress of biofilms grown on different coated plates was similar. Moreover, the figure illustrates that the biofilms grown on uncoated stainless steels seem to be the easiest to clean. However, the exact shear stress that results in visually clean surfaces could not be obtained. This is because a sudden removal of biofilms was observed at the stress beyond 8 Pa for all the biofilms samples. This removal stress corresponds to a shear stress caused by liquid flow in pipe of a flow rate of around 2 m/s (Equation 2); this flow rate is the typical flow rate used in Cleaning-In-Place procedures for industrial processing equipment.

Effect of roughness

The sample plates used in the system of Arthronema africanum biofilms were all stainless steels with mechanical treatments so that their roughness varied. Table 4 lists the roughness of these sample plates, which was measured as mean square roughness (Ra).

Figure 6 illustrates biofilms formed on tested plates and it is obvious that mechanical treatments affect the amount of biofilms formed on the surfaces. A clear trend was also observed from the figures: the smoother the surface, the larger the amount of biofilms formed. As biofilm formation generally starts with adhesion of cells to surfaces (Bryers, 2000), the smooth surface here may not be smooth enough to prevent this adhesion. Comparing sizes of *Arthronema africanum* cells - which are narrow and cylindrical in shape, with a width of 0.8-5 µm and up to 5 times that in length (Wehr and Sheath, 2003) - with the roughness of the electropolished plate (Table 4), adhesion of Arthronema africanum cells are possible. Furthermore, the change from attached cells to biofilm is triggered by the conditions causing stress

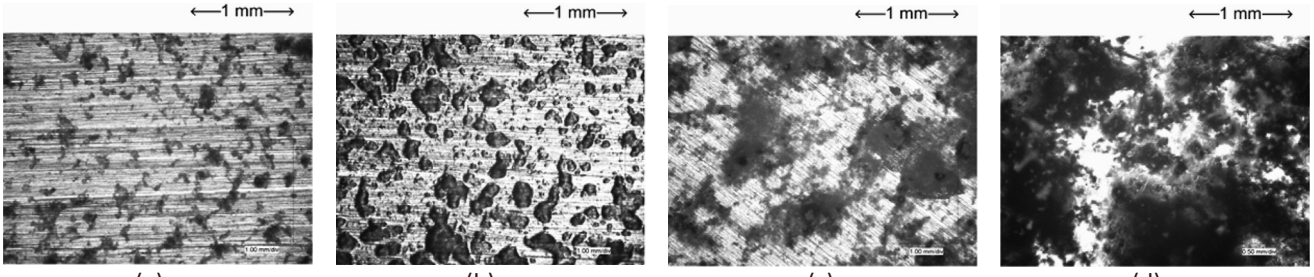

Figure 6. Micrograph of biofilms deposited on (a) untreated stainless steel and mechanical treated stainless steels namely (b) K80; (c) K240; (d) electropolished: Scale bar shows 1 mm.

(e.g. limited nutrients, being washed away by flows) to microorganisms (Toutain et al., 2004); cells attached to smooth surface are exposed to higher shear stresses caused by flowing liquid and hence more biofilms are observed on the smoother surface.

Nevertheless, the surface roughness shows insignificant effects on the removal stress, which is the shear stress required for cleaning sample plates to the point of visual cleanliness. The removal stress measured using FDG for all the sample plates was in the same range at approximately 46 Pa. Figure 7 depicts the cleaned surfaces of the K 240 roughened and electropolished plates, which were the two plates covered with observable amount of biofilms. The cleaned areas were not clean at microscopic level but there was no variation in cleanliness between the two surfaces.

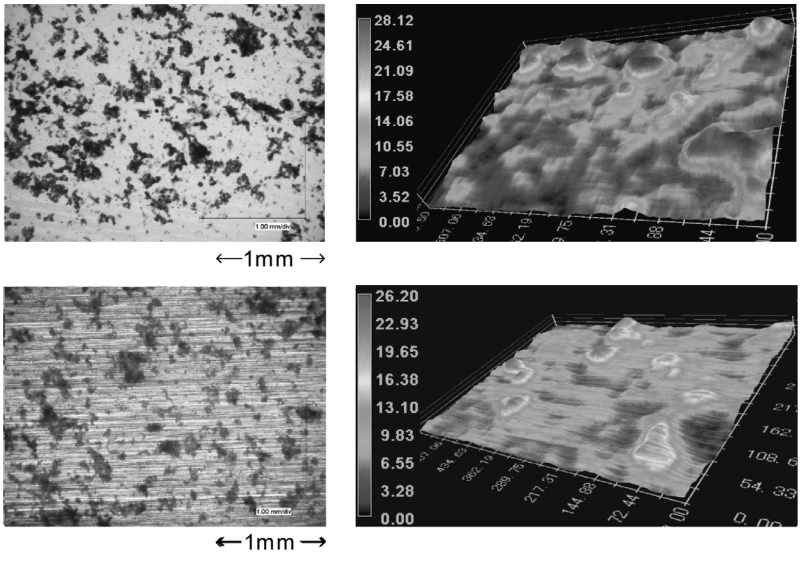

Figure 7. Micrograph of surfaces in the cleaned region after biofilm removal by FDG: (a) K240, 2D image; (b) K 240, 3 D image (600x600 μm, height scale of 26.20 μm; (c) Electropolished, 2D image; (d) Electropolished, 3D image (600x600 μm, height scale of 28.12 μm).

4. Applications of the Findings

Concluding from the results in this paper, a new method of preventing formation of biofilms on building exteriors would be to have smooth exterior surfaces. Since Gaylarde and Gaylarde (2005) have classified building exteriors with biofilms to two types, the applications of this study were based on these two types of exteriors: (i) mineral substrate (e.g. stone) and (ii) painted substrate (e.g. painted metal, painted wood).

4.1 Protective Coatings for Mineral Substrate

Recently, anti-soiling coatings, especially self-cleaning ones, have become a focus of interest in the field of architectural coatings (Zielecka and Bujnowska, 2006). These coatings will be suitable for use with the mineral substrate type of building exteriors; when applied, the polymer (mostly silicone) would fill in naturally occurring gaps on mineral substrates and produce smooth coated surfaces. Different types of these coatings, with varying properties, are available: for example, hydrophilic coating, which increases cleanability (Nagaya, 1996). These properties were obtained by means of engineering the surface energy of the coatings. This can be done by adding other polymers to the silicone coatings. Nevertheless, none of this matters in the prevention of biofilm formation, as the effects of surface energy observed in this study on the formation process were negligible. Moreover, a simple

silicone coating is considerably smoother than the surfaces studied here (168 nm – 242 μm, compared with the ~ 50 nm reported by Zielecka & Bujnowska, 2006). Hence, it would be sensible to suggest that simple silicone coatings would be sufficient for biofilm formation.

4.2 Paint formulation for painted substrates

Gaylarde and Gaylarde (2005) demonstrated that biofilms could form even on the paint films which contained biocides. This was because the duration of modern antifungal biocide activity in external paint films is short compared to the lifetime of the film itself (Gaylarde et al., 2004). However, concluding from the results in this paper, new paint formulation should focus on the surface roughness of the finished paint films rather than the types and amounts of biocides incorporated in them. For example, Tiarks et al. (2003) suggested that by formulating paint from a paste, less pigment clustering occurred, resulting in a smoother finished paint film.

5. Conclusion

Preliminary works have shown that surface properties influence the formation and cleaning of biofilms. In this study, significant effects of surface roughness were observed compared to the effects of surface materials (or surface energy). Biofilms tended to form more on smooth surfaces but these biofilms were as easily cleaned as those formed on rougher surfaces. However, the results were derived from conditions where the surfaces were always exposed to liquid. Hence, further investigation should be done in conditions where the surfaces are simply in contact with moisture in the atmosphere to simulate the context of building exteriors. Furthermore, it should be noted that surface roughness will also affect the local water content of building exteriors. Hence, in addition to having an effect on initial cell adhesion, the surface roughness will affect the local conditions at the surfaces, which are other crucial factors to biofilm formation.

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