

APPLICATION OF FLUID DYNAMIC GAUGING IN THE CHARACTERISATION AND REMOVAL OF BIOFOULING DEPOSITS

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ABSTRACT

Green cleaning is generally defined as cleaning of a surface by consuming minimal resources in order to lessen the impact on human health and environmental quality. The main aim of this study is to perform cleaning studies of *Escherichia coli* biofilms grown on (i) polyethylene, (ii) stainless steel, and (iii) glass, to observe their removal behaviour under controlled hydrodynamic conditions. The biofilms grown on the three different substrates were tested using the technique of fluid dynamic gauging (FDG), which allows for the estimation of the cohesive (within the biofilm structure) and adhesive (between biofilm and substrate) strength of the deposits. The results show that the thickness of biofilm on all substrates increases with time and plateaus at 14 days. Mature biofilms grown on glass have a stronger surface attachment than those on stainless steel and polyethylene. The results also suggest structural weakening after 21 days, implying that cells have died.

INTRODUCTION

All forms of fouling have the potential to be hugely detrimental to industrial processes, especially when found on pipelines and heat transfer surfaces. Biofouling is no exception. The problem is most notable in the food industry, where biofilms can grow on all surfaces that are vulnerable to local bacteria inhabitation, such as pipe bends, conveyor belts, floors and rubber seals (Blanchard et al. 1998). As well as the process equipment, microbes can also thrive on ceilings (as a result of condensation), gutters and drains of food processing facilities. The pharmaceutical, oil and petrochemical industries are also affected, as are water transportation networks. The increased costs associated with biofouling can be attributed to many factors. These include interference with the process itself, deterioration of the product (quantity or quality), damage to process materials and hardware, and shortened life of components due to cleaning (Flemming et al. 2011). Cleaning of equipment may require

temporary shutdown of the relevant section, resulting in inferior productivity.

The organic polymers which form the overall biofilm matrix are known as extracellular polymeric substances (EPS). These substances are produced and excreted by the micro-organisms present, and their chemical structure varies in accordance with the organisms which produce them, as well as being influenced by environmental conditions (Momba et al. 2000). Organic matter accumulates on the surface which allows for colonisation by a small number of cells. This initial attachment is reversible, and may initially be the rate limiting step of the entire process. The subsequent stage is irreversible attachment, which is initiated by the production of EPS strengthening the bond with the surface (Yebra et al. 2006). Jiao et al. (2010) showed that mature biofilms can contain more than twice as much EPS than those midway through development. Features attributed to EPS include the formation of a gel-like network keeping the bacteria together, the mediation of adherence of biofilms to surfaces, and the protection of bacteria against noxious influences from the environment. One of the most important functions of extracellular polysaccharides is their role as fundamental structural elements of the EPS matrix determining the mechanical stability of biofilms (Wingender et al. 1999).

Biofilms have developed many different defence mechanisms against disinfection, to the extent that biofouling is a problem which can effectively never be eliminated. The success of antifouling measures are therefore time-dependent rather than permanent – without regular disinfection, bacterial colonisation of surfaces will continue to progress (Flemming et al. 2011). For some disinfectants, the concentration must be increased by between 10 and 100 times in order to achieve the same level of deactivation of biofilm-based bacteria compared with their planktonic equivalents (Blanchard et al. 1998). This is partly due to the EPS, which is important for attachment and resistance to natural shear forces, and also owes much to the physiological adaptation to existence within a biofilm (sessile growth, nutrient stresses,

continuous contact with low levels of disinfectant) (Bridier et al. 2011). The boundary layer effects of the adjacent surface also play a part, especially in laminar regimes where the boundary layer is substantial enough to restrict the movement and mixing of cells (Donlan 2002). Chlorine has long been used as a disinfectant in water-based systems, although survival and multiplication of micro-organisms is often observed even after a regular, consistent supply. With antibacterial substances only a control of the symptoms of the infection is possible, the biofilms remains and will support rapid regrowth (Momba and Makala 2004).

There are certain alternative or additional methods available for the removal of biofilms. For instance, mechanical removal can be used in which the principles of fluid dynamics can be applied. Early investigations observed increasing detachment at higher applied shear forces and greater flow velocities. Investigations into removal patterns relative to flow velocities and wall shear stresses are of huge importance, given that care should be taken to reduce chemical consumption. Furthermore, in terms of energy use, doubling the flow velocity requires four times as much pumping power (Lens et. al. 2003). It is therefore essential that the optimum method of removal is known.

The study of biofilms poses a set of additional challenges. Firstly, deposits found in the food and biotechnology industries are known to regularly contain prominent liquid fractions. Methods involving the use of a probe or similar device would most likely lead to inaccurate measurements as the fragile deposit layer would be expected to deform upon contact. Also, deposits which contain a biological component invariably shrink or slump outside of their natural environment. There is also tendency for considerably variation in the properties of the biofouling within a single sample. These parameters dictate that the gauging method must not involve physical contact with the deposit surface, be operable in situ, and be adaptable to conduct 'local' measurements to account for variability. Traditional gauging methods encounter difficulties in these areas. Analysis of the electrical conductivity of the sample requires contact with the surface which makes it unsuitable. Others, such as ultrasound or silicon sensors, whilst they can be carried out in situ, would require prior knowledge of the deposits via preliminary steps. Optical methods, typically confocal laser scanning microscopy (CLSM), can indicate biofilm thickness using cell staining, although this is too expensive for regular use and is carried out ex-situ. Pressure drop analysis would appear to meet the criteria, but offers only an average thickness measurement across the sample.

Various methods have been utilised in the studying of biofouling, with some success. Most notably, Fowler and McKay (1980) developed the radial flow cell (RFC) to study the impact of shear forces on the growth and development of biofilms, and this has recently been used to assess the removal of different deposits from solid surfaces. Demilly et. al. (2006) used the RFC to study the detachment kinetics of yeast from a stainless steel substrate, specifically the effects of the surface topography. The

major drawback of the RFC is that in situ observations are generally not possible, and furthermore only one point of interest can realistically be analysed per sample. Tuladhar et al. (2000) proposed fluid dynamic gauging (FDG), a variation on pneumatic gauging, as a solution which meets the necessary criteria.

Fluid Dynamic Gauging

In this study FDG is employed to investigate the removal behaviour of biofilms from three substrates, namely polyethylene, glass and stainless steel. As well being a non-contact method, FDG requires little prior knowledge of the physical properties of the deposit, which can be complex and time consuming to determine. The hydrodynamic conditions can be controlled in a relatively simple manner, which both manipulates the experimental variables and prevents the invasion of any foreign matter. It allows the thickness of the fouling deposits to be determined, and the adhesive and cohesive strengths in resistance to shear can also be measured. A schematic of an FDG nozzle in proximity to a fouled surface is shown in Figure 1.

The process fluid is siphoned into the nozzle using a fixed pressure drop between the surrounding fluid and at the discharge point after it has been drawn through the nozzle. The resultant flow rate can then be measured. This method is entitled 'mass flow mode'. An alternative method is to run FDG in 'pressure mode', which involves measuring the pressure drop across the nozzle operating under a constant flow rate, which is useful for high pressure systems or where a consistent gauging flow is desirable (Gu et al. 2011). Mass flow mode is used in this work.

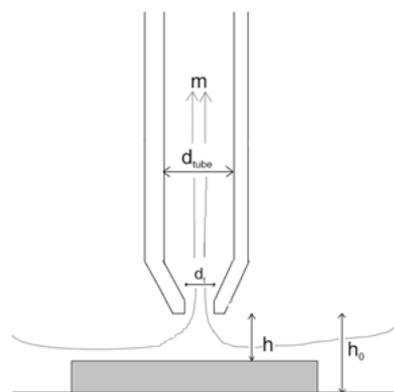


Fig.1: A schematic of the FDG nozzle in proximity to a test surface, where h is the distance between the nozzle and the deposit surface, h_0 is the distance between the nozzle and the clean surface, d_i is the nozzle diameter, d_{tube} is the diameter of the siphon tube, and m is the fluid mass flow rate.

The key operational variable is the dimensionless value of h/d_i , the ratio of the nozzle clearance distance to the internal diameter of the nozzle. The shear stress acting on the surface due to gauging flow depends on the flow conditions and h/d_i . The imposed shear stress, τ_w , can be determined using computational fluid dynamics (CFD).

For low h/d_t values, Chew et al. (2004) show that it can also be appropriately approximated by the equation which represents the radial flow between two parallel discs:

$$\tau_w = \frac{3\mu m}{\rho\pi h^2} \frac{1}{r} \quad (1)$$

where τ_w is the wall shear stress, μ is the dynamic viscosity of the fluid (in this case water), ρ is the density of the fluid, and r is the radial distance from the central axis of the nozzle. CFD simulations showed that the maximum wall shear stress occurs directly underneath the inner rim of the nozzle. So the maximum shear stress imposed on the gauged surface is calculated using Eq. (1) when $r = d_t/2$.

Thus far, Möhle et al. (2007) have demonstrated the ability of FDG to measure the adhesive strength of biofilms grown on sandblasted polycarbonate discs by quantifying the applied shear stress using the model of laminar flow between parallel discs. Furthermore, the cohesive strength of biofilms and EPS was analysed using FDG by Otto (2008) by way of monitoring the thickness of the biofilm at different stages in the removal process. Salley et al. (2012) showed how FDG operated using liquid expulsion can monitor the removal of biofilms on polyethylene and stainless steel surfaces, and suggested the existence of a two-tier structure - a compact layer adjacent to the surface and a loose upper layer.

The primary focus of this research is to investigate the application of 'green cleaning' ideas into varied industrial settings, predominately in the production of food and pharmaceuticals. The term green cleaning is broadly defined as "cleaning to protect health without harming the environment" (Green Cleaning Network 2012), concerning both the products and equipment used, and also the associated policies and responsibilities tasked with protecting human health and the environment. The other major aspect of green cleaning is the pursuit of sustainability – the use of resources in a controlled way which preserves them for future generations. These factors combine to complete a picture in which reductions are sought in the amount of chemicals, water and energy used in cleaning. As mentioned above, FDG is a technology which utilises hydrodynamic phenomena to measure the thickness and strength of fouling layers. The result of this, with respect to green cleaning, is that the strength of deposits can be tested and the optimum water usage estimated. This allows for the design of effective cleaning protocols which minimise energy usage and environmental impacts.

FDG was utilised in mass flow mode (Chew et al. 2004) in order to study the removal behaviour of bacterial biofilms (in this case *Escherichia coli* Nissle1917) from three surfaces, namely polyethylene, glass and stainless steel 304 grown for a range of incubation periods. Results were obtained using a combination of microscopic techniques and analytical methods.

MATERIALS AND METHODS

Bacteria Strains and Culture Media

Three strains of bacteria were tested for their ability to form biofilms. These were as follows: *Escherichia coli* Nissle1917; and two variants of *Pseudomonas aeruginosa* (PA01 and NCTC). Cultures were grown in M9 minimal medium (De Kievit et al. 2001), containing 47.7mM $\text{Na}_2\text{HPO}_4 \cdot 7\text{H}_2\text{O}$, 21.7mM MKH_2PO_4 , 8.6mM NaCl , 18.7mM NH_4Cl , 0.5% (wt/vol) Casamino acids, 1mM MgSO_4 and 11.1mM glucose (all sourced from SigmaAldrich).

Biofilm Assay

Each strain was cultured overnight at 37°C, and then diluted in fresh media to an optical density (OD600) of 0.06. The diluted cultures were added to wells of a 96-well polyethylene microtitre plate, with a row of four plates dedicated to each strain plus another row of pure medium for control purposes. The plate was then incubated at 37°C on an incubator (Stuart Mini Gyro-Rocker SSM3) rotating at maximum speed (70 rpm). Cells attach to the bottom of the wells and form a biofilm. After 24 h the supernatant fluid was pipetted out and replaced with another 200µL of fresh medium. Incubation was resumed for another 24h period. In order for the biofilm growth to be quantified, the wells were washed with 0.9% saline and stained with crystal violet (Bolej et al. 2011). The absorbance of the content of each well (including the control wells) were measured and recorded using an automatic plate reader (VERSAmax Tunable Plate Reader BN 02877) at a wavelength of 595 nm, as wavelengths in the region of 600 nm are a good option for most bacterial cultures, with the advantage that the media components contribute less to the overall absorbance than at lower frequencies (Burton and Kaguni 1997).

Confocal Laser Scanning Microscopy

Images of biofilms grown for a period of 5 days were taken using CLSM (with an LSM 510META microscope). Samples were washed with 0.9% saline and stained using 0.1% acridine orange solution. These images proved particularly useful for observing growth on the stainless steel discs, for which optical microscopy proved to be unsuitable.

FDG Testing

Figures 2(a) and (b) show the apparatus used in this study. The only difference being that the nozzle in (b) is geometrically five times larger than that of (a). The FDG nozzle is held normal to the surface, and the pressure profile altered as a result of a change in proximity to the surface. The two fluid dynamic gauges were run (under mass flow mode) in order to conduct biofilm removal experiments. The different nozzle diameters (1 mm in (a) and 5 mm in (b)) allow different ranges of shear stresses to be applied (see Eq. (1)). The nozzle used in Figure 2(a) can apply shear stresses of approximately between 25 and 150 Pa, whilst the nozzle in 2(b) offers a range of 2 – 60Pa.

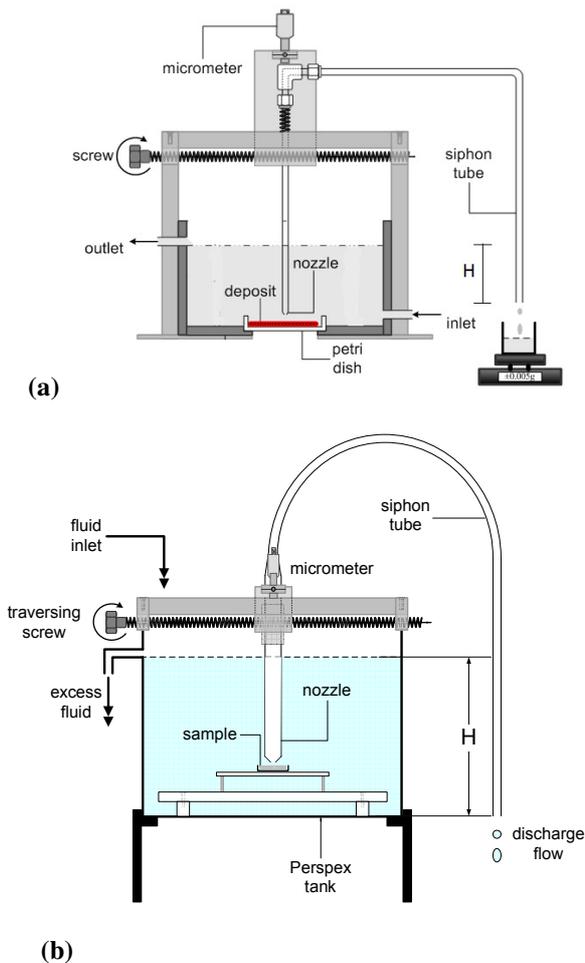


Fig.2:(a) A labelled schematic of the small static FDG rig, where H is the hydrostatic head providing the siphon driving force. Nozzle diameter (d_t) = 1 mm (Sahoo et al. 2008). (b) The portable FDG rig. The feed tube was connected to a tank fed by a nearby tap, and the weir maintained a consistent water level (Chew et al. 2004).

Biofilm strength tests. The protocol of incubation followed the same method for growth as detailed for the assay, with the exception that in this case three different substrates were selected for comparison: (i) polyethylene petri dishes; (ii) glass petri dishes; and (iii) stainless steel 304 discs. A range of incubation periods set at 5, 10, 14, 21 and 28 days was chosen as a means of assessing the stages of growth and development. Separate samples were grown for each of the time periods. It is not possible to re-use the samples once a strength or thickness test has been performed. Before the FDG process, imaging was performed using a Nikon Eclipse E400 optical microscope in order to provide images of the fouled surface prior to removal.

The samples were then placed under the gauge at different nozzle clearance heights (h/d_t) to impose a range of shear stresses in order to test the yield shear strength of the biofilm deposits. Four tests were carried out on each sample, each test at a different h/d_t value. Three repeats of each h/d_t value were conducted. After FDG was applied to

the samples, the surfaces were again analysed under the microscope at each gauged point, and the percentage reduction in surface coverage measured using *ImageJ* (developed for the public domain by the US National Institutes of Health). The requirement of the optical microscope dictated that the cultures grown on stainless steel could not be analysed in this way, as the cells were not visible due to background effects.

The biofilm removal evident in the images can also be related to the shear stresses imposed by the gauging flow and calculated using Eq.(1), and henceforth to the equivalent mean pipe flow velocity using Eq.(2):

$$U_m = \sqrt{\frac{2\tau_w}{\rho C_f}} \quad (2)$$

where C_f is the fanning friction factor and U_m is the mean pipe flow velocity. For turbulent flow regimes, C_f is typically equal to 0.005.

Biofilm thickness tests. Similar to the strength tests, biofilms were grown on all three surfaces for periods of 5, 10, 14, 21 and 28 days. Measurements of biofilm thickness were taken from each, using the flow data from the FDG experiments in comparison with calibration data taken using clean surfaces. These tests were conducted with the aim of assessing the cohesive strength of the biofilm structures and the evolving maturity of the EPS matrix over time.

RESULTS AND DISCUSSIONS

Biofilm Assay: Strain Comparison

The mean absorbance of the control samples was subtracted from the values for the biofilm wells, and mean results for each strain were calculated and displayed in Figure 3. The *E.coli* Nissle1917 sample exhibited more extensive biofilm attachment than both *P. aeruginosa* strains for the conditions applied (which were kept constant throughout), and was thus grown for all subsequent investigations.

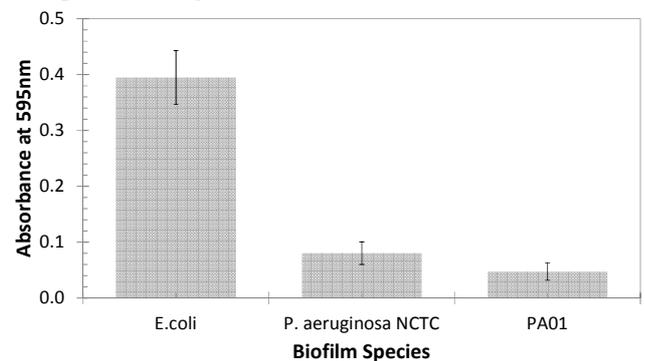


Fig. 3: Mean absorbencies for each biofilm species (with standard deviations shown as error bars). Biofilms were grown in 48-well microtitre plates using M9 minimal media. Four samples were grown of each strain, along with blanks for control purposes.

Confocal Laser Scanning Microscopy

The images in Figure 4 were captured using the CLSM 510META. Laser images of the biofilm growth on the steel discs (5-day incubation) were taken in conjunction with z-stack analysis of the surface at different elevations in order to produce a projected image of the film thickness. The images show a moderate coverage of stained cells, with evidence of clusters of biofilm, noticeable due to both an increased density of cells and surrounding background stain which may be due to extracellular polymeric substances (EPS). This shows evidence of *E. coli* biofilm growth on stainless steel surfaces, with potential for further growth after a longer period of incubation.

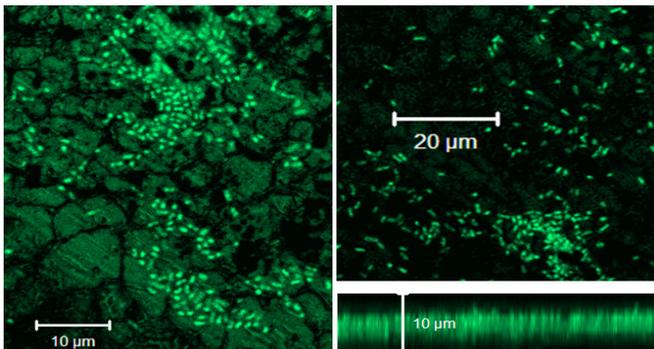


Fig. 4: CLSM images of two separate stainless steel discs with *E. coli* biofilms grown for 5 days and a ‘z-stack’ of the first disc (bottom right) showing the thickness of the biofilms present.

Adhesive Strength Tests

Figure 5 shows an example of strength test results obtained from biofilms grown for 5 days on polyethylene. As the figure from part (a) to part (d), the shear stress applied is increased, and removal of biofilm from the substrate accelerated accordingly. The result indicates that a shear stress of approximately 18 Pa is necessary to eliminate the biofilm from polyethylene.

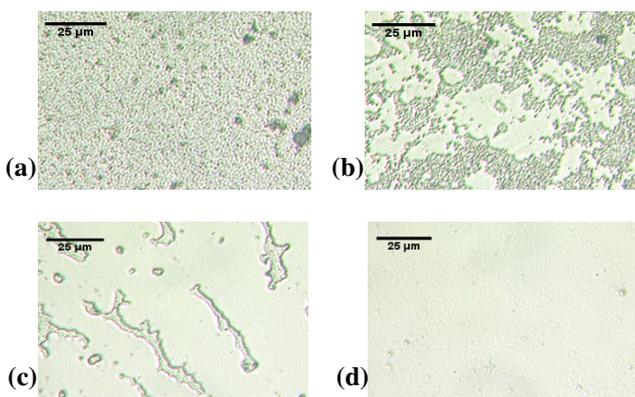


Fig. 5: Nikon AZ100 optical microscope images of biofilms incubated for 5 days on polyethylene surface: tested under FDG at (a) $h/d_t = 0.3$, (b) $h/d_t = 0.25$, (c) $h/d_t = 0.2$, (d) $h/d_t = 0.15$. Shear stress values, τ_w , at each stage calculated using Eq. (1): (a) 6.3 Pa, (b) 9.4 Pa, (c) 12.6 Pa, (d) 18.2 Pa.

Figure 6 shows an equivalent strength test on a glass surface – in this case the shear stress required for complete removal was 16 Pa. The most significant difference from the results shown in Figure 5 is the readiness with which the biofilm is sheared off. Approximately 72% of the glass surface is cleaned after the application of 6.5 Pa (Image (b)), whereas for the polyethylene surfaces, approximately twice as much stress is required to remove the same amount.

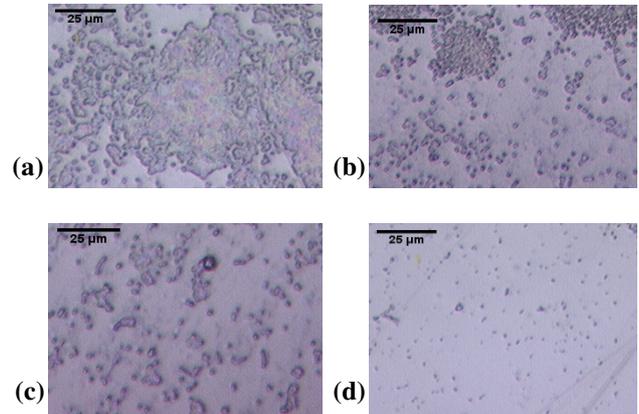


Fig. 6: Optical microscope images of biofilms incubated for 5 days on glass surface: tested under FDG at (a) $h/d_t = 0.23$, (b) $h/d_t = 0.19$, (c) $h/d_t = 0.15$, (d) $h/d_t = 0.11$. Shear stress values, τ_w , at each stage calculated using Eq. (1): (a) 4.7 Pa, (b) = 6.5 Pa, (c) 9.8 Pa, (d) 16.1 Pa.

Figure 7 is an interpolation showing the estimated mean pipe flow velocity (calculated using Eq. (2)) required to remove 95% of the biofilm surface coverage plotted against the incubation times. At 10 days' incubation, biofilms grown on polyethylene were stronger than those grown on glass (3.2 m/s pipe flow required to eliminate polyethylene based biofilms, 2.4 m/s to remove from glass). The removal behaviour was reversed after 14 days, where the attachment to the glass dishes has become considerably stronger (4.8 m/s required), whilst the polyethylene samples are more readily removed with a flow of 3.1 m/s. This would imply a stronger, or more consistent, initial attachment of *E. coli* biofilms to polyethylene and an earlier maturation. It is possible that, once initiated, the glass-based biofilms become more firmly established despite experiencing difficulties in the early stages. A decline in adhesive strength was noticeable on both surfaces after growth periods of 21 and 28 days despite the daily provision of fresh medium, suggesting the weakening of EPS structures (due to degradation of components important for adhesion, usually polysaccharides (Ahimou et al. 2007)) and/or the death of cells. On both surfaces, the biofilms which were incubated for 14 days proved the hardest to remove, closely followed by the 10-day biofilms. These results suggest that cleaning *E. coli* biofilms from glass surfaces should be relatively more straight-forward, provided it is carried out regularly before the biofilms become established.

For polyethylene surfaces, however, initial attachment is more rapid and therefore more energy would be required to clean them where sterility is an important factor. The strong early attachment of bacteria to polyethylene is followed by an earlier maturation and hence a more rapid weakening of bonds, and dispersion. In related literature it has been suggested that the first stage may be the rate-limiting step for the entire culture development process (Momba et al. 2000).

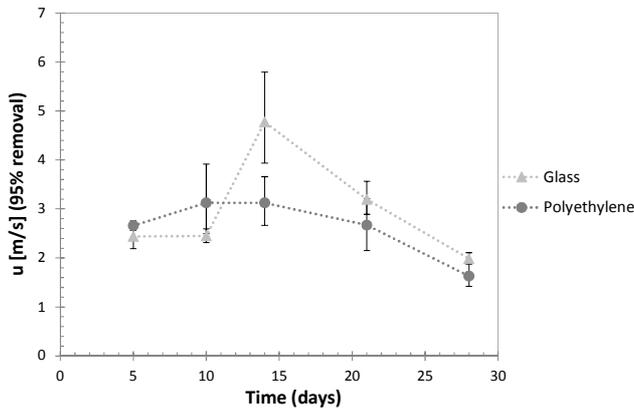


Fig. 7: The estimated mean pipe flow velocity required to achieve 95% removal of biofilm coverage for glass and polyethylene surfaces for a range of incubation times. The error bars relate to the potential inaccuracy of the interpolation, and the scope for experimental errors.

Thickness and Cohesive Strength Tests

The average values for the original thickness of biofilms grown are displayed in Table 1. It is worth noting that the errors in the measurements indicate significant variations in the growth behaviour. These results suggest a consistent, rapid increase in thickness up to the 14-day growth point, followed by the beginnings of a slow natural reduction once the biofilms have fully matured. In general, the table shows strong similarities between the thicknesses experienced across the surfaces, suggesting that whilst the substrate may impact upon the ability of biofilms to attach, it has little effect on the potential thickness of the deposits.

Table 1: The average thickness of biofilms grown on all three substrates over the range of incubation periods, with error margins.

Incubation period (days)	Polyethylene [μm]	Glass [μm]	Stainless Steel [μm]
5	9 ± 4	13 ± 6	13 ± 3
10	41 ± 10	68 ± 16	47 ± 13
14	109 ± 21	148 ± 30	128 ± 22
21	123 ± 22	127 ± 38	116 ± 43
28	107 ± 30	104 ± 16	95 ± 18

Whilst image analysis is helpful in quantifying the surface coverage (i.e. the adhesive strength of biofilms);

the cohesive strength between layers is also of importance and can be quantified using thickness reduction tests. Generally, the relationship between thickness reduction and velocity in this section echo the results from the strength tests. The biofilms grown for 5 and 21-28 days were the easiest to remove, whilst those grown for 10 and 14 days proved to be more resilient. The trend was less clear for the glass-based biofilms, in which removal rates were similar with the exception of the notable cohesive strength of the 14-day biofilms. Figure 8 assimilates these results to show the patterns, with the same method as with Figure 7 from the previous section.

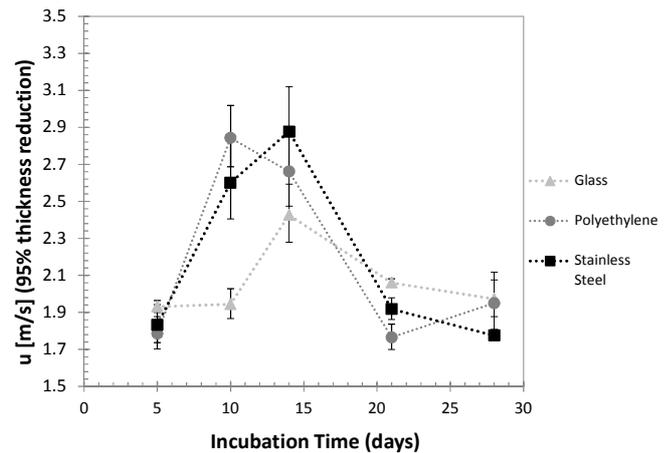


Fig. 8: The estimated mean pipe flow velocity required to remove 95% of biofilm thickness from different surfaces for a range of incubation periods.

Figure 8 shows that the strength peak for polyethylene occurs after 10 days (approximately 20 Pa), and for glass the strength of 5-day and 10-day biofilms is comparable (9-10 Pa), the point for 14 days appearing to be the beginning of its peak – a value of approximately 15 Pa, which is also incidentally the lowest peak of the three substrates. The results in Figure 8 also supports the concept of the attachment, maturation and dispersion stages of biofilm development, and bears a resemblance to the graph for the strength tests in Figure 7. The first phase (initial and irreversible attachment) is analogous with the fragile attachment seen after the 5-day incubation. Maturation seem to occur at approximately the 10-14 day period with peaks as high as 20-21 Pa (or 2.8-2.9 m/s) for polyethylene and steel. By the time biofilms had spent 21 – 28 days in incubation, growth on all three substrates would appear to have reached the dispersion phase, accounting for the relative ease of removal at lower shear stress. Older biofilms are also at risk from sloughing, which is the tendency of large portions of biofilm to detach suddenly under shear.

The best recommendation at this stage for a cleaning protocol would be to clean the fouled surfaces when the deposits are at their weakest. This indicates that cleaning after either 5 or 21 days would result in the easiest removal. If the decision was made to clean after 21 days of

growth, this would carry a greater risk of contamination due to the opportunity for the dispersal of pathogenic cultures. The relative ease of removal at this stage implies that sections of biofilm can readily detach from the bulk deposit, and it is logical due to the findings of these experiments to suggest that this may regularly occur under normal process flow conditions. On the other hand, the greater regularity of cleaning after every 5 days would incur greater energy and water requirements. A cleaning protocol would therefore be dependent on the industry in question (for example, food production may demand the regular cleaning option due to the enhanced dangers of biological contamination).

Furthermore, the levels of wall shear stress required for effective biofilm removal is more comparable to those employed in cleaning-in-place (CIP) systems, which operate under turbulent flow, despite the FDG process utilising laminar flow for removal. The shear stress levels required for biofilm removal indicate the necessity for turbulent flow in a larger scale system, and the corresponding fluid velocities are unjustifiably high in accordance with green cleaning principles. Therefore, it is clear that the input of cleaning additives or enzymes will be required. In summary, the optimisation of water and chemical consumption has not yet been achieved, but the findings from the FDG technique so far are likely to be crucial in establishing any greener cleaning protocol.

CONCLUSIONS

The *E. coli* strain successfully formed biofilms grown under static conditions suitable for analysis on the three substrates, namely glass, polyethylene and stainless steel. Typical maximum thicknesses were in the region of 100 – 140µm, which was true for all surfaces tested. FDG has been successful in indicating the yield strength of biofilm adhesion and strength of bacterial cohesion within biofilms over a range of incubation periods. Results suggest a relationship between maturity and biofilm strength with a peak in cohesion after approximately 14 days, and a weakening of structures thereafter. This suggests that biofilms could be removed with minimum energy required either between establishment and 5 days' growth, or after more than 21 days' growth, taking into account the relevant costs and risk of product contamination. The method which best combines efficiency with green cleaning ideals is likely to require the use of some chemical detergents or enzymes due to the pumping power duties necessary for a solely mechanical cleaning protocol being unsuitably high.

NOMENCLATURE

C_f	Friction coefficient	-
d_t	Gauge nozzle diameter	m
d_{tube}	Gauge tube diameter	m
h	Nozzle-deposit distance	m
h_0	Nozzle-clean surface distance	m

m	Mass flow rate	kg/s
r	Radial distance from nozzle centre	m
U_m	Mean pipe flow velocity	ms ⁻¹
μ	Fluid viscosity	Pas
ρ	Fluid density	kg/m ³
τ_w	Wall shear stress	Pa

Abbreviations

CIP	Cleaning-in-place
CLSM	Confocal Laser Scanning Microscopy
EPS	Extracellular Polymeric Substances
FDG	Fluid Dynamic Gauging
RFC	Radial Flow Cell

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