Journal of Applied Microbiology 1999, **87**, 41–48

Effectiveness of cleaning techniques used in the food industry in terms of the removal of bacterial biofilms

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7107/02/99: received 30 October 1998, revised 26 February 1999 and accepted 4 March 1999

H. GIBSON, J.H. TAYLOR, K.E. HALL AND J.T. HOLAH. 1999. The effectiveness of cleaning was investigated through food factory trials and laboratory experiments using a naturally occurring biofilm from a food factory environment and generated biofilms. The efficacy of factory cleaning and disinfection programmes was assessed by swabbing and total viable count (TVC) analysis of surfaces before cleaning, after cleaning and after disinfection. Cleaning produced a 0·91 log reduction in the attached population. Investigation of the effectiveness of a variety of cleaning methods in the removal of a naturally occurring food factory biofilm showed that the high pressure spray and the mechanical floor scrubber, which use a high degree of mechanical action, were most effective. Cleaning trials with biofilms of *Pseudomonas aeruginosa* or *Staphylococcus aureus* showed that spraying with water at pressures of 34·5, 51·7 and 68·9 bar did not significantly increase the removal, as assessed by direct epifluorescent microscopy (DEM) and swabbing and TVC analysis, beyond the three log reduction observed at 17·2 bar. The effect of spray time at 17·2 bar showed that increasing spray time from 1 to 10 s did not significantly increase removal of *Ps. aeruginosa* biofilm. Investigation of the optimum distance of the spray lance from the surface at 17·2 bar was found to be between 125 and 250 mm. The use of an alkaline, acidic or neutral detergent prior to spraying with water at 17·2 bar did not significantly increase the removal of *Ps. aeruginosa* or *Staph. aureus*. However, the acidic and alkaline products significantly ($P = 0{\cdot}05$) affected the viability of *Staph. aureus* and *Ps. aeruginosa*, respectively, thereby minimizing the potential for the spread of contamination.

INTRODUCTION

Bacterial attachment to surfaces and biofilm formation are well recognized phenomena in a variety of environments such as marine, freshwater, medical, food and other industrial systems (Carpentier and Cerf 1993; Zottola and Sasahara 1994). A biofilm consists of surface-colonizing microbes and associated polymers. In the food processing environment, the conditions favour attachment and biofilm formation, i.e. flowing water, suitable attachment surfaces, ample nutrients (although possibly sporadic) and raw materials, or the environment supplying the inocula. Attachment of a variety of organisms to food processing surfaces has been reported by a number of workers (Herald and Zottola 1988; Holah *et al.* 1988, 1989; Frank and Koffi 1990; Krysinski *et al.* 1992; Kim and Frank 1994) with associated changes in the biocide sensitivity (Best *et al.* 1990; Frank and Koffi 1990; Holah *et al.* 1990; Lee and Frank 1991; Dhaliwal *et al.* 1992; Mosteller and Bishop 1993; Ronner and Wong 1993). The formation of biofilms has also been widely reported in food processing environments (Holah *et al.* 1989; Holah and Kearney 1992; Mattila-Sandholm and Wirtanen 1992; Carpentier and Cerf 1993; Zottola and Sasahara 1994; Gibson *et al.* 1995). The time available for biofilm formation will depend on the frequency of cleaning regimes. Product contact surfaces may typically be cleaned several times per day, while environmental surfaces such as walls may be cleaned once per week. There is, therefore, more time for biofilm formation on environmental surfaces. Gibson *et al.* (1995) found that although attachment to a variety of surfaces in the food processing environment readily occurred, extensive surface

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colonization and biofilm formation only occurred on environmental surfaces. Product contact surfaces may contaminate the product directly as the product touching or passing over the surface will potentially pick up microbial contamination. Environmental surfaces such as floors and walls may be indirect sources of microbial contamination that can be transferred to the product by vectors such as the air, personnel and cleaning systems (Holah 1992; Holah *et al.* 1993). The hygiene of the surfaces therefore affects the quality and safety of the food product.

Provided that the equipment and environment are hygienically designed (i.e. with no crevices, dead spaces etc.), an effective cleaning and disinfection (sanitation) programme is the major method of control of the surface route of contamination. If the sanitation programme is not effective, micro-organisms and product residues will remain at concentrations that may affect the quality and safety of the food product.

The sanitation programme removes undesirable material (or soil) from the surfaces, including micro-organisms, product residues, foreign bodies and cleaning chemicals. This involves a number of stages: wetting of the soil and surface by the cleaning chemical, reaction of the chemical to facilitate removal from the surface, prevention of re-deposition and disinfection of residual microbes (Jennings 1965; Koopal 1985; Holah 1992). There are four factors involved in the sanitation programme that are used in combination to achieve the stages described above: chemical energy, mechanical/kinetic energy, temperature/thermal energy, and time. Chemical energy is important for the cleaning and disinfection phases. In the cleaning phase, the chemicals break down soils and reduce their attachment strength to facilitate removal from the surface. In the disinfection phase, the chemicals reduce the viability of the microbes remaining after cleaning. Mechanical or kinetic energy is employed to remove soils from the surface physically and may include manual brushing, scraping, automated scrubbing, pressure-jet washing or the circulation of fluid in clean-in-place systems. Temperature affects cleaning and disinfection in several ways. Firstly, the chemical effects increase linearly with temperature. Secondly, a temperature above the melting points of fat and oils facilitates their removal although high temperatures can increase the tenacity of protein soils due to the denaturing of the protein. The time component can be increased by the use of soak tanks, foams or gels to increase the contact time between the chemical and the soils on the surface.

The cleaning phase is thought to be the most important stage for minimizing microbial colonization and removing attached micro-organisms (Carpentier and Cerf 1993; Dunsmore 1981). However, cleaning chemicals are developed for the removal of particular types of soils (e.g. fat, starch, protein and mineral salt deposits) rather than micro-organisms. In addition, the cleaning phase is generally designed and optimized in terms of food product soil removal.

The aim of this study was to evaluate the role of the cleaning and disinfection stages of open surface sanitation programmes in the removal of bacterial biofilms and to evaluate the effectiveness of different cleaning parameters in terms of the removal of bacterial biofilms.

MATERIALS AND METHODS

Bacterial strains and media

Previous studies had shown that pseudomonads and staphylococci are particularly prevalent in food processing environments (Gibson *et al.* 1995). *Pseudomonas aeruginosa* (NCIMB 10421) and *Staphylococcus aureus* (NCTC 10788) were used. Cultures were maintained by long-term storage by freezing at -20 °C using Cryobeads (Lab M, Bury, UK). Weekly master cultures were prepared by adding a bead to 150 ml nutrient broth (Oxoid) in a conical flask and shaking at 30 °C overnight (16 h). Working cultures were prepared by adding a 1 ml aliquot of the master culture to 150 ml nutrient broth and incubating at 30 °C overnight (16 h).

Test surfaces

The surfaces used were type 316 stainless steel coupons (10×4 cm) with a 2B finish. The surfaces were prepared by washing in mild detergent (SU121, Diverseylever, Annesley, UK), rinsing in tap water and sterilizing by autoclaving at 121 °C for 15 min

Biofilm formation in the laboratory

Bacterial suspensions were prepared by centrifuging the overnight cultures at 3600 g for 10 min. The pellets were re-suspended in phosphate buffer (34 g potassium dihydrogen phosphate l^{-1} distilled water, pH 7·2). The stainless steel coupons were immersed in the bacterial suspension for 1 h at room temperature to allow attachment. The bacterial suspension was then removed and replaced with growth medium (1·0 g bacteriological peptone and 0·7 g yeast extract l^{-1} distilled water) and incubated at room temperature for 4 h. After this period, an incomplete monolayer biofilm of either *Ps. aeruginosa* or *Staph. aureus* had developed on the surfaces (approximately 10^7 cells cm^{-2}).

Biofilm formation in a factory environment

The ducting of a factory blancher extractor system was found to promote extensive biofilm formation on the inner surfaces of the ducting system. In order to allow study of naturally occurring biofilms, stainless steel coupons (prepared as described above) were placed inside the inspection hatches and left in place for 5 d. After 5 d, an extensive, mixed culture,

multi-layered biofilm had developed on the surfaces. The surfaces and attached biofilms were then used in cleaning studies. The nature of the biofilm was not characterized.

Swabbing and total viable count determination

The surfaces were swabbed ($5 \times 4\,cm^2$) with cotton-tipped swabs pre-moistened with diluent (1·0 g bacteriological peptone and 8·5 g sodium chloride l^{-1} distilled water) and placed in a 10 ml volume consisting of 9 ml diluent and 1 ml inactivator (3 g soya lecithin, 30 ml Tween-80, 5 g sodium thiosulphate, 1 g L-histidine and 10 ml phosphate buffer l^{-1} distilled water, pH 7·2). The organisms present were resuspended from the swab by vortexing for 30 s. The resuspension fluid was serially, decimally diluted in diluent and duplicate 1 ml aliquots were removed for pour-plating using nutrient agar (Oxoid). Plates were incubated at 30 °C for 2 d. This technique was used to quantify the number of organisms present on a surface after a particular treatment, and was also used to assess the surface population before cleaning, after cleaning and after disinfection in a 10 week trial in each of eight factories. Field trials were only undertaken in factory environments where the concentration of bacteria on the surfaces before cleaning was approximately 10^6 cfu $swab^{-1}$ so that the effect of cleaning and disinfection could be quantified. Holah *et al.* (1988) evaluated the effectiveness of swabbing as a method of assessing surface populations and found that swabbing removed a constant proportion of organisms from the surface above surface populations of 10^5 cfu cm^{-2}.

Direct epifluorescent microscopy (DEM)

The stainless steel surfaces with attached bacteria were stained with 0·1 mg ml^{-1} acridine orange (Difco) for 2 min at room temperature. The surfaces were then gently rinsed in sterile distilled water to remove non-attached organisms, allowed to air dry and stored in the dark until examination. The attached population was enumerated using an epifluorescence microscope (Olympus BH2 Olympus Optical Co. (UK) Ltd., London, UK) linked to an Optimax V image analyser (Synoptics Ltd, Cambridge, UK). In the cleaning studies using factory-generated biofilms, the mean percentage area covered by orange fluorescence was assessed in 20 fields of view. This percentage area covered on surfaces with monoculture laboratory-generated biofilm was converted to cells cm^{-2} using a calculation based on the mean size of 20 measured individual organisms. The typical sizes of *Ps. aeruginosa* and *Staph. aureus* were 1·45 and 0·61 μm^2, respectively; however, the size was checked before counting each set of stainless steel surfaces as certain treatments, such as detergents, reduced the cell size.

Cleaning chemicals and treatments

Cleaning chemicals. The detergents used in this study were chosen to represent those used in the food industry. The following detergents were used: Easyclean (an alkaline detergent, pH 11·6, used at 5% from Maigret Chemicals, Daventry, UK), Ambersan (an acidic detergent, pH 1·7, used at 2·5% from Tampen and Tampen Ltd., Fordingbridge, UK), SU121 (a neutral detergent, pH 8·3 used at 1% from Diverseylever, Annesley, UK) and Shuregel no. 2 (used at 1–5% from Diverseylever, Annesley, UK). The contact time used was 20 min.

Pressure washing. A KEW system (KEW Cleaning Systems Ltd, Penrith, UK) was used to spray the surfaces at 17·2, 34·5, 51·7 and 68·9 bar. In addition, the surfaces were sprayed using the KEW system without the pump switched on to give a pressure (i.e. mains pressure) of 7·0 bar. Surfaces were held in a purpose-built rig, which allowed the time of spraying, and the distance of the nozzle from the surface, to be controlled. The nozzle of the lance was routinely placed 200 mm from the surfaces and surfaces were sprayed for 5 s with the water jet at an angle of 90° to the surface. Mains water at ambient temperature was used for all experiments.

Mechanical action. A floor scrubber was used to assess the effect of mechanical action on the removal of naturally occurring factory biofilms. Surfaces were mounted in a purpose-built rig and cleaned using a floor scrubber for 5 s with a rotating scrubber brush moving in one direction across the surface.

Statistical analysis

In most of the experiments, triplicate stainless steel surfaces were used for each treatment and the experiments were performed on three separate occasions. In the studies examining the efficiency of the different cleaning techniques against naturally occurring factory biofilms, four replicate samples were used. Differences in the number of bacteria remaining on the surfaces after each particular treatment were assessed statistically by analysis of variance (MINITAB Statistical Software, Minitab Inc., Pennsylvania, USA); significance is expressed at the 95% confidence level.

RESULTS

The results in Table 1 show that in the factory environment, cleaning produced a 0·91 (log mean) or 1·18 (arithmetic mean) log reduction, while the disinfection phase produced a slightly greater log reduction (1·54 arithmetic, 1·21 log mean). Laboratory cleaning trials investigated the effect of high pres-

Table 1 Mean concentration of bacteria on surfaces in eight factories before cleaning, after cleaning and after disinfection as determined by swabbing and TVC analysis

	Before cleaning TVC (cfu swab^{-1}) ($n = 498$)	After cleaning TVC (cfu swab^{-1}) ($n = 1090$)	After disinfection TVC (cfu swab^{-1}) ($n = 3147$)
Arithmetic mean	$1{\cdot}32 \times 10^6$	$8{\cdot}67 \times 10^4$	$2{\cdot}50 \times 10^3$
Standard deviation	$2{\cdot}42 \times 10^7$	$1{\cdot}10 \times 10^6$	$4{\cdot}41 \times 10^4$
Log arithmetic mean	6·12	4·94	3·40
Mean Log	3·26	2·35	1·14
Standard deviation	1·80	1·65	1·31

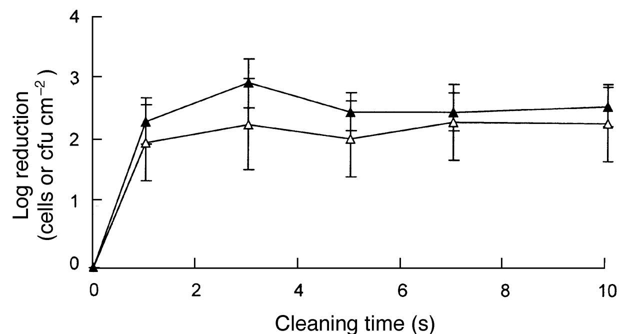

Fig. 2 Effect of high pressure/low volume spray time at 17·2 bar on the removal of *Pseudomonas aeruginosa* biofilms as determined by TVC (closed symbol) and DEM (open symbol). Results are mean values from three separate experiments ± S.E.

sure/low volume spray pressure, spray time, distance of the spray lance form the surface and the effect of detergents on the removal of biofilms. Figure 1 shows the effect of spraying *Ps. aeruginosa* and *Staph. aureus* biofilms on stainless steel surfaces with water at different pressures. The log reductions achieved show that there was a three log reduction in the surface population with spraying. The results based on TVC analysis and DEM counts show very similar trends. A good correlation between TVC and DEM was obtained for surface concentrations between 10^3 and 10^7 cells cm^{-2}. The number of organisms removed did not significantly increase with increasing pressure.

Figure 2 shows the effect of high pressure spray cleaning time on the removal of attached *Ps. aeruginosa*. Cleaning times above 1 s did not significantly increase the removal of the bacterial biofilm. The effect of distance of the spray lance from the surface on the removal of *Ps. aeruginosa* biofilms is shown in Fig. 3. The results show that the optimum distance for removal is between 125 and 250 mm. Figures 4 and 5 show the effect of detergents on the removal and viability, respectively, of attached *Ps. aeruginosa* and *Staph. aureus*. There was no significant difference between the effectiveness of the detergent products in terms of the removal of attached *Staph. aureus* (Fig. 4a), with 2 log reductions for all three products (i.e. greater than 10^4 cells cm^{-2} remained on the surfaces). In addition, spraying at pressures above 17·2 bar did not significantly increase the removal of the *Staph. aureus* biofilm. The data in Fig. 5(a) show the effect of the detergents on the viability of attached *Staph. aureus*. The acidic product produced the highest log reductions (approximately 6 log orders) and there was no significant difference between the alkaline and neutral products (log reduction 3–5).

The removal of *Ps. aeruginosa* (Fig. 4b) was similar to that observed for *Staph. aureus* (approximately 3 log orders). In addition, there was no significant difference between the

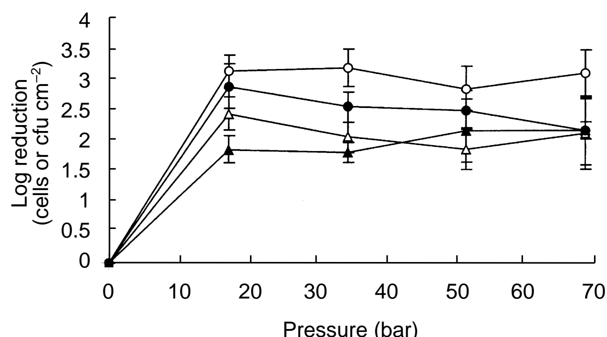

Fig. 1 Effect of high pressure/low volume water spray on the removal of *Pseudomonas aeruginosa* (▲) and *Staphylococcus aureus* (●) biofilms as determined by TVC (closed symbol) and DEM (open symbol). Results are mean values from three separate experiments ± S.E.

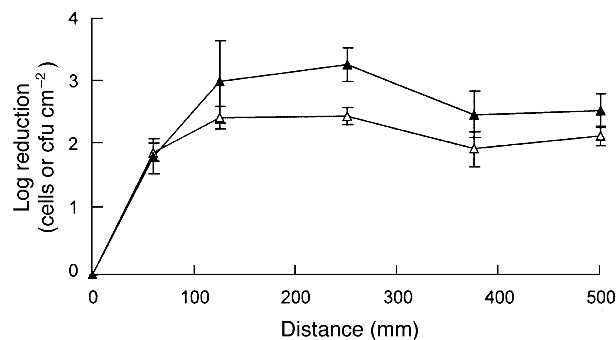

Fig. 3 Effect of the distance of the high pressure/low volume spray lance from the surface on the removal of *Pseudomonas aeruginosa* biofilms at 17·2 bar as determined by TVC (closed symbol) and DEM (open symbol). Results are mean values from three separate experiments ± S.E.

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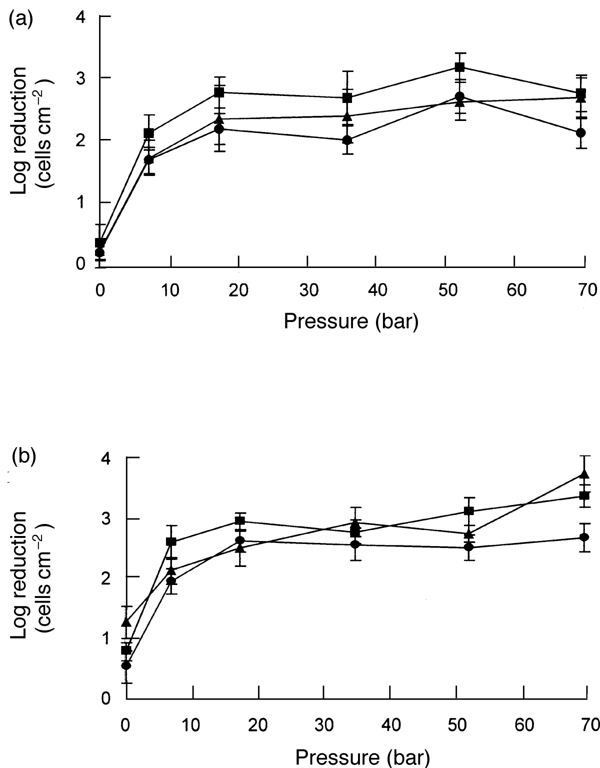

Fig. 4 Effect of detergents (acidic ▲, alkaline ■, neutral ●) and high pressure/low volume spray on the removal of *Staphylococcus aureus* (a) and *Pseudomonas aeruginosa* (b) as determined by DEM. Results are mean values from three separate experiments ± S.E.

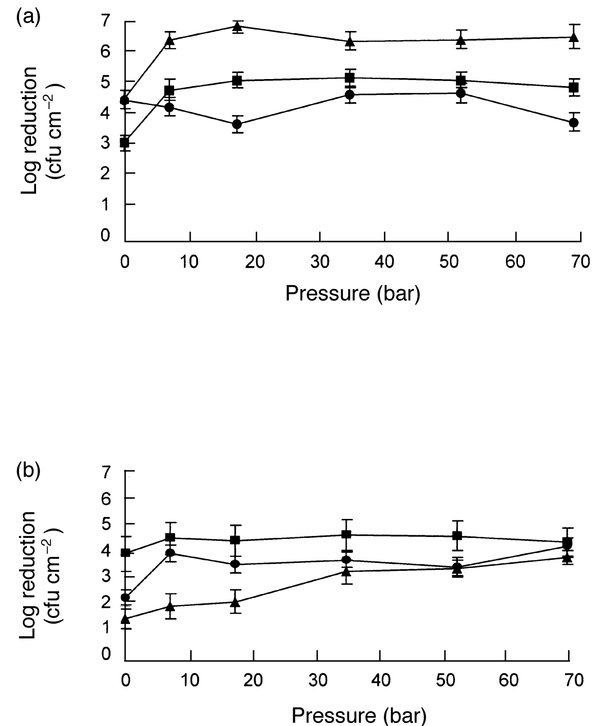

Fig. 5 Effect of detergents (acidic▲, alkaline ■, neutral ●) and high pressure/low volume spray on the removal of *Staphylococcus aureus* (a) and *Pseudomonas aeruginosa* (b) as determined by TVC. Results are mean values from three separate experiments ± S.E.

effectiveness of the detergents in terms of the removal of *Ps. aeruginosa*. Figure 5(b) shows the effect of the detergents on the viability of *Ps. aeruginosa*. In the case of this organism, the alkaline product was the most effective, producing log reductions of approximately 4–5 log orders, although the log reductions were generally lower for *Ps. aeruginosa* than *Staph. aureus*. Comparison of the results in Figs 1, 4 and 5 shows that the detergent did not significantly increase cell removal over spraying with water alone.

Table 2 compares the efficiency of a range of cleaning methods in terms of the removal of a naturally occurring biofilm. The results show that there was 98% coverage on the untreated control biofilms with a TVC of $1{\cdot}7 \times 10^8$ cfu cm^{-2}. The application of the gel, followed by a low pressure rinse, resulted in a small decrease in coverage but had little effect on the viability of the attached population. The cleaning routine performed by the factory personnel was relatively ineffective, as there was no significant reduction in the area coverage or TVC. The mechanical floor scrubber reduced the area coverage to less than 1% and the TVC to $6{\cdot}6 \times 10^5$ cfu cm^{-2}. The high pressure spray also reduced the area coverage to less than 1% and the TVC to $8{\cdot}9 \times 10^4$ cfu cm^{-2}.

Table 2 Comparison of the effectiveness of a range of cleaning techniques in terms of removal of the factory generated biofilm assessed by DEM assessment of area covered and swabbing and TVC analysis

Cleaning treatment	Mean percentage area coverage (S.D.)	Mean TVC (cfu cm^{-2}) (S.D.)
Control untreated biofilms	98·4 (3·4)	$1{\cdot}7 \times 10^8$ ($5{\cdot}0 \times 10^6$)
Gel detergent plus low pressure rinse	82·7 (18·5)	$1{\cdot}6 \times 10^8$ ($3{\cdot}4 \times 10^7$)
Mechanical floor scrubber	0·4 (0·4)	$6{\cdot}6 \times 10^5$ ($6{\cdot}7 \times 10^5$)
High pressure spray wash	0·1 (0·0)	$8{\cdot}9 \times 10^4$ ($7{\cdot}6 \times 10^4$)
Factory clean (low pressure rinse, disinfection, rinse)	95·1 (3·6)	$1{\cdot}5 \times 10^8$ ($5{\cdot}0 \times 10^6$)

DISCUSSION

The factory trials were only undertaken in factory environments where the concentration of bacteria on the surfaces

before cleaning was approximately 10^6 cfu swab^{-1} so that the effect of cleaning and disinfection could be quantified. The results from the factory environments before cleaning, after cleaning and after disinfection show that the cleaning phase of the sanitation programme is responsible for the removal of approximately 1 log order of micro-organisms from surfaces. However, Dunsmore *et al.* (1981) found that the cleaning phase removed 99·8% (i.e. an almost 3 log reduction) of the milk soil and bacteria present on stainless steel surfaces. The cleaning phase is important for efficient disinfection as it removes the product soils, which could potentially inactivate the disinfectant or protect the micro-organisms from disinfectant action. These results show that although cleaning reduces the concentration of micro-organisms on surfaces, significant numbers of micro-organisms remain on surfaces after cleaning and therefore, the disinfectant stage is still required. The fact that 10^3 cfu swab^{-1} remained after cleaning shows that there is scope for optimization of cleaning regimes in terms of the removal of attached micro-organisms.

Removal of *Ps. aeruginosa* and *Staph. aureus* biofilms was not enhanced significantly with increasing water spray pressure. This is an important observation as spraying at higher pressures significantly increases aerosol generation. Holah *et al.* (1990, 1993) showed that high pressure systems generated aerosols that could potentially disperse viable micro-organisms over an extensive area. In addition, the aerosol droplets generated by high pressure systems are generally smaller and may therefore remain suspended for longer periods of time. In terms of the removal of bacteria, 17·2 bar is sufficient as increasing pressure does not enhance removal; the use of this lower pressure may also limit the potential spread of contamination. Higher pressures may still be required for the removal of particular types of product soils. It is important to note that 10^4 cells cm^{-2} remained on the surfaces after the spray treatments and confirms the requirement for a subsequent disinfection step.

High pressure cleaning times above 1 s did not significantly increase the removal of the bacterial biofilm. The kinetic energy imparted through the impact of the water droplets on the surface was probably responsible for the removal, rather than water running across the surface, as longer cleaning times would have increased the removal if this was the case. This probably means that the use of a pressure lance alone will result in the patchy removal of bacteria and possibly soil from the equipment surface as all areas will not be exposed to a 1 s spray with the water jet. The use of a detergent could facilitate the removal of the soils and microbes from the surface, which can then be rinsed away by the water spray.

The optimum distance of the spray lance from the surface for the removal of bacterial biofilm was between 125 and 250 mm. The reason for the differences in removal at different distances may be due to the droplet sizes impacting on the surface and associated differences in kinetic energy imparted at the surface. Although the removal at 125–250 mm was statistically significantly greater than other distances, the effect of distance over this range was within 1 log order. For practical purposes, therefore, there is a wide range of acceptable distances that a spray lance can be held from the surface by a cleaning operative to ensure satisfactory cleaning performance.

Detergents are generally formulated to remove particular types of soils, for example, proteinaceous, fatty, carbohydrate or mineral soils, rather than to remove micro-organisms. The detergents studied did not significantly improve the removal of attached *Ps. aeruginosa* and *Staph. aureus* and there was no difference in terms of extent of removal between the Gram-positive and Gram-negative organisms. This shows that these detergents do not enhance the removal of bacterial biofilms. Similarly, Wirtanen *et al.* (1995) found that the use of a detergent had a limited effect on the cleanability of biofilms from surfaces in the absence of any product debris or other organic soiling. The role of detergent in removal of bacteria from surfaces may have more significance in the presence of food debris where in addition to direct attachment to the stainless steel surface, micro-organisms may be attached to food particles, the removal of which would be facilitated by appropriate detergents.

Although there was no difference between the detergents in terms of removal, the acidic detergents and the alkaline products produced significant reductions in the viability of *Staph .aureus* and *Ps. aeruginosa*, respectively. The removal of *Staph. aureus* was only approximately 3 log orders, and the acidic detergent reduced the viability of the remaining bacteria so that only 1 log order remained. Consequently, this cleaning process was particularly effective at reducing the viable population of attached *Staph. aureus*. In contrast, *Ps. aeruginosa* was more resistant to the detergent products so that a maximum of 4 log reductions was observed and, therefore, greater than 3 log orders remained on the surfaces after cleaning. Dunsmore *et al.* (1981) found that an acidic product was more effective than an alkaline product in terms of effect on cell viability. This trend was observed for *Staph. aureus* but not *Ps. aeruginosa*. Lewis *et al.* (1989) found that higher pH values resulted in greater removal of *Acinetobacter* species with about 100 times as many bacteria detached at pH 12 as pH 2. Similarly, Czechowski (1990) found that alkaline products were more effective at detaching biofilms, and chlorinated alkaline detergents were more effective than non-chlorinated alkaline detergents. The difference in the effectiveness of detergents against these two organisms may relate to their differing colonization mechanisms. Bacteria attached to surfaces produce extracellular material that is often poly-anionic in nature and forms a matrix around the cells which can protect the cells from adverse conditions. The amount and nature of the polymers produced by micro-organisms varies between species (Beech *et al.* 1991; Spenceley *et al.*

1992; Wirtanen and Mattila-Sandholm 1992) and may relate to the differences in the effectiveness of the detergents. These data demonstrate the importance of choosing an appropriate and effective detergent. Detergents that aid removal of attached bacteria and simultaneously reduce the viability of those organisms in the process have added benefits in terms of minimizing the generation of aerosols of viable micro-organisms. As the biofilm may consist of a mixed population of a variety of Gram-positive and Gram-negative organisms, investigation of the nature of the biofilm could facilitate the choice of an effective product and may require the use of a combination of detergents.

The evaluation of the efficiency of a range of cleaning methods in terms of the removal of a naturally occurring biofilm showed that the application of the gel followed by a low pressure rinse resulted in a small decrease in coverage but had little effect on the viability of the attached population. The use of the detergent and the low pressure rinse may have removed or destabilized the extracellular material surrounding the attached population, thereby reducing the area covered without affecting the viable count. Cations and, in particular, calcium are thought to play a role in the bonding of polymer molecules in the biofilm. Removal or absence of cations results in detachment (Camper *et al.* 1993). Chelators present in detergents may therefore play an important role in biofilm destabilization and subsequent removal. The cleaning routine performed by the factory was relatively ineffective as there was little reduction in either the TVC or area covered. It was particularly difficult to gain access to the extractor system and this is perhaps the reason for the relatively poor clean. The mechanical floor scrubber was very effective, reducing the area coverage to less than 1% and the TVC to approximately 10^5 cfu cm^{-2}. The mechanical energy involved in this technique was particularly effective in the removal of attached micro-organisms and biofilms. Similarly, the high pressure spray wash, which also utilizes a high level of kinetic energy, reduced the area coverage to less than 1%. Similarly, Mattila-Sandholm and Wirtanen (1992) reported that mechanical cleaning is the most efficient way to remove attached micro-organisms and biofilm. Mechanical action in the form of brushing has been shown to be effective by other workers (Exner *et al.* 1987; Holah *et al.* 1990). Although the high pressure spray and the mechanical floor scrubber were particularly effective, it is important to consider the possible spread of contamination by these cleaning techniques. High pressure spray systems, in particular, produce aerosols of viable micro-organisms that could, potentially, be dispersed over a wide area (Holah *et al.* 1993).

In conclusion, there is scope for increasing the importance of the cleaning phase in terms of the removal of attached bacteria. This can be optimized in terms of the efficacy of removal and limitation of the generation of viable aerosols by using a method that provides a high degree of mechanical action in conjunction with detergents that reduce cell viability. This study suggests that detergents may play a role in the reduction in the spread of contamination by aerosols. However, further work is required to optimize this effect.

ACKNOWLEDGEMENTS

This work was funded in part by the Ministry of Agriculture Fisheries and Food.

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