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Removal of Bacterial Biofilms

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SUMMARY

Bacteria attach to almost any surface and can develop into extensive biofilms. The surfaces of the food processing environment may be a direct or indirect source of contamination. The food product may pick up contamination as it moves across product contact surfaces. Alternatively, contamination on environmental surfaces may be transferred to product by vectors such as personnel, pests, air movement or cleaning systems. Cleaning and disinfection regimes are the major controls of this surface route of contamination.

This study investigated the efficacy of cleaning techniques and chemicals in terms of the removal and disinfection of bacterial biofilms. The particular techniques and parameters were selected to be typical of those used in the food industry, based on results of surveys of the industry. The parameters investigated included spray water pressure, water temperature, distance of the spray lance from the surface, spray cleaning time, gel and foam contact time, detergent type, presence of soil and cleaning technique. The study investigated the effectiveness of these techniques and parameters in terms of removal and disinfection of attached organisms.

CONTENTS

INTRODUCTION

A biofilm consists of microbes and a matrix of extracellular products in association with a substratum. The matrix consists largely of water (98-99% according to Christensen and Characklis, 1990) and various polymers, commonly polysaccharides and glycoproteins. The microorganisms are not uniformly distributed throughout the biofilm as the biofilm consists of microcolonies with channels in between, resulting in a rather heterogeneous structure (Geesey *et al*, 1992).

Microbial cells attach firmly to almost any surface in a wide range of natural environments; for example, microbes are found on almost all surfaces in marine and freshwater systems with algal biofilms developing on any illuminated surface which is submerged in water or in a humid environment (Leadbetter and Callow, 1992). Transmission and scanning electron microscopic (SEM) studies have shown that immobilised cells grow, reproduce and synthesise extracellular polymers which frequently extend from the cell, forming an extracellular matrix (Characklis, 1990). The absorptive nature of this matrix means that particles may be trapped and inorganic components may bind to the matrix. Sessile or attached microbial populations exist on surfaces in flowing systems and are often present in higher numbers than the planktonic population, particularly in oligotrophic systems (Blenkinsopp and Costerton, 1991). Biofilms range from monolayers of cells to the complex mixtures found in algal mats which may be up to 300-400mm thick. Biofilms are ubiquitous in nature and the organisms often exist as members of complex consortia, rather than pure cultures. Their localised metabolic activity can create diffusion gradients of nutrients, fermentation by-products and other products associated with corrosion, resulting in a mosaic of micro-environments that may be completely different to the bulk phase (Keevil *et al*, 1990).

Bacteria gain a number of advantages from the biofilm mode of growth; eg, microbial cells attached to stones in a stream are protected from ultraviolet rays. (Frank and Koffi, 1990). Cells in biofilms are also protected from other hostile environments; for example, biofilm organisms are protected from preservatives, disinfectants, antibiotics and biocides (Mattila-Sandholm and Wirtanen, 1992) and antibacterial agents and heat (Frank and Koffi, 1990) to a greater extent than freely suspended organisms. Many antibiotics work by inhibiting active growth, and as biofilm cells have a different growth rate, they do not exhibit the same sensitivity to antibiotics as their planktonic counterparts (Gilbert *et al*, 1990). Biofilms therefore constitute a reservoir of many different species able to resist environmental fluctuations. Moreover, the polysaccharide fibres in the organic polymeric matrix, which are generally negatively charged, trap the organic and mineral molecules and particles in the bulk phase (Carpenter and Cerf, 1993).

Problems associated with biofilms can cause significant financial loss to industry, e.g. increased fuel costs due to the fouling of ship hulls and heat transfer reduction in power station heat exchangers. In addition biofilms can pose health problems; for example, large amounts of biofilm may slough off the walls of water distribution pipes causing the concentration of cells to rise above levels considered safe for consumption. Biofilms in water distribution systems may act as a reservoir of potentially pathogenic organisms as the biofilm organisms may be resistant to even high levels of chlorine (Block, 1992). The primary mode of dispersion of *Legionella pneumophilia* is in aerosols and often occurs in cooling water towers, and once attached to surfaces *L.pneumophilia* is far more resistant to biocides (Wright *et al*, 1991). Biofilms may also form on medical implants such as catheters, resulting in extremely recalcitrant infections (Stickler and Hewett, 1991).

The importance of biofilms in food processing environments has, to date, received little attention. In food processing environments, microbial contamination of the food product may arise from four main sources: the constituent raw materials, surfaces, people (and other animals) and the air.

The cleanliness of the surfaces of the equipment and the environment affect the quality and safety of the food product, which are related to the presence of spoilage microbes and pathogens respectively.

The food product may pick up contamination as it is moved across product contact surfaces or if it is touched by food handlers or pests. The air acts as both a source of contamination from outside the food processing environment or as a transport medium moving contamination from non product to product contact surfaces (Holah and Kearney, 1992).

Food processing environments provide a variety of conditions which might be expected to favour the formation of biofilms, i.e. flowing water, suitable attachment surfaces, ample nutrients (although possibly sporadic) and raw materials or the environment supplying the inocula. The time available for biofilm development is usually relatively short as some production lines may operate for as little as an hour, although others may run for several days. Biofilm development in food processing environments can have detrimental effects on the microbial status of the food product. Biofilms may harbour a variety of organisms, including pathogens that can contaminate the product through direct contact or indirectly via vectors such as people, pests, air movement or cleaning systems.

Undoubtedly, improved technology in the production, manufacture and distribution of food has led to considerable improvements in hygiene, but at the same time the increases in the scale of production and the scope of distribution open the possibility of larger and more widespread food poisoning incidents. In fact the incidence of food poisoning has continued to increase over recent years, emphasising the requirement for further improvements in hygiene.

Cleaning and disinfection are undertaken to remove all the undesirable material (food residues, microorganisms, foreign bodies and cleaning chemicals) from the surfaces to a level such that the residues remaining are of minimal risk to the safety and quality of the product (Holah, 1992). Cleaning and disinfection is therefore the major control of the surface route of contamination. When undertaken correctly, cleaning and disinfection regimes are a cost effective way of reducing the risk of microbial and foreign body contamination. This is becoming increasingly pertinent due to the intrinsic demands for higher standards of hygiene required for the production of short shelf life chilled foods and preservative-free products.

Biofilms protect microorganisms from being washed away in the product flow, from cleaning and disinfection and, in sites that dry out, from desiccation. Biofilm organisms can be more resistant to antibacterial agents. Holah *et al* (1990a) showed that attached organisms may be 100 times more resistant to disinfectants commonly used in the food industry. Similarly Le Chevalier *et al* (1988) demonstrated that biofilm organisms were 150-3000 times more resistant to hypochlorous acid than were unattached cells. Frank (1990) found that *Listeria* species grow in the food processing environment within multi-species biofilms and Frank and Koffi (1990) found that *Listeria monocytogenes* in a biofilm was resistant to several disinfectants. There are two ways in which biofilms could result in contamination of the product. Firstly, persistent contamination may be due to a failure of the cleaning regime to remove biofilm which can quickly regenerate to act as a source of contamination. Swabs of the main product contact surfaces may fail to detect these hidden reservoirs of infection. Secondly, the biofilm may be the vehicle for spreading contamination from one piece of equipment to another. The cleaning regime often generates aerosols of bacteria and debris and these aerosolised biofilm fragments may be better protected and survive longer.

Biofilms have been found on a variety of product contact and non-product contact or environmental surfaces (Table 1a and b). Microbial attachment to heat exchanger plates in cheese and liquid milk factories is a well known source of bacterial contamination of dairy products (Bouman *et al*, 1984), and Zoltai *et al* (1981) used scanning electron microscopy to demonstrate the adhesion of bacteria to the inside of a milk storage tank. Lewis and Gilmour (1987) also investigated the adhesion of the milk flora to transfer pipes made of rubber and stainless steel. Holah *et al* (1989), using stainless steel coupons and direct epifluorescent microscopy (DEM), found that microcolonies rapidly developed on both an egg glaze and a buttermilk line and that multilayered biofilms formed on the surfaces of a baked bean production line. Environmental surfaces may also harbour bacteria that may be transferred to product by people, pests or cleaning systems. Water is often liberally used in many operations so that static surfaces such as floors may receive intermittent but regular flows of dilute nutrients and consequently the microbial loading in such environments can be extremely high. Other studies conducted at the Research Association have identified food processing environments which allow biofilm development to occur and types and levels of microorganisms found on food contact and non-food contact surfaces (Gibson *et al*, 1995).

The aim of this study was to investigate the efficacy of cleaning techniques and chemicals in terms of the removal and disinfection of bacterial biofilms. The particular techniques and parameters were selected to be typical of those used in the food industry based on results of surveys of the industry. (Appendix II)

TABLE 1(a)

SUMMARY OF THE LITERATURE REPORTS ON MICROBIAL ADHESION AND BIOFILMS IN THE FOOD INDUSTRY

Product	Time[1]	Count	Method	Reference
Raw milk	20h	-	SEM	Zoltai *et al* (1981)
Raw milk (inoculated)	2-6h 12h	0 10^4 cfu/cm^2	Swab	Bouman *et al* (1984)
Pasteurised milk (inoculat ed)	2-6h 12h	Organisms present 10^6 cfu/cm^2		
Milk	5 days	$8.1x10^3$ mesophiles/cm^2 (stainless steel) $3.5x10^4$ mesophiles/cm^2 (rubber) $8.51x10^3$ psychrotrophs/cm^2 (stainless steel) $8.6x10^4$ psychrotrophs/cm^2 (rubber)	Squeegee rinse	Lewis and Gilmour (1987)
Ham slicing	-	10^5 mesophilic aerobes/cm^2	Double swab	Bizzaro *et al* (1990)
Poultry	-	-	SEM	Notermans *et al* (1991)
Abbatoir smoked salmon	- -	10^4 cfu/cm^2 10^4 cfu/cm^2	Swab	Spenceley (1993)
Baked beans transport belt Egg glaze bath Fish filleting Buttermilk in margarine production	2-4h 6-8h 12h 16h 0-2h 2-4h 4-6h 6-8h 0-2h 2-4h 4-6h 6-8h 0-2h 2-4h 4-6h 6-8h	$2.1x10^6$ cells/cm^2 $1.1x10^7$ cells/cm^2 $>1.7x10^7$ cells/cm^2 $>4.3x10^7$ cells/cm^2 $8.0x10^4$ cells/cm^2 $2.0x10^4$ cells/cm^2 $9.0x10^4$ cells/cm^2 $1.7x10^6$ cells/cm^2 $4.0x10^4$ cells/cm^2 $2.3x10^4$ cells/cm^2 $1.3x10^4$ cells/cm^2 $3.4x10^3$ cells/cm^2 $2.3x10^4$ cells/cm^2 $1.8x10^4$ cells/cm^2 $1.8x10^4$ cells/cm^2 $8.1x10^5$ cells/cm^2	DEM	Holah *et al* (1989)

[1] Time a cleaned surface was exposed to product

TABLE 1(b) - SUMMARY OF AREAS WHERE BIOFILMS WERE DETECTED (GIBSON *et al*, 1995)

Area	Exposure Time (hr)	Count/cm²	% Coverage	Product
Waste can area	24	$\geq 3.5 \times 10^7$	15.0	Canned product
Blancher extractor	24	$\geq 4.7 \times 10^7$	26.5	Canned products
	24	$\geq 4.2 \times 10^7$	23.8	
	24	-	20.2	
	48	-	66.3	
	72	-	85.2	
	120		98.4	
Mixer - under surface of ledge	20	$\geq 5.9 \times 10^7$	56.1	Meat substitute
Ceiling	24	5.5×10^5	3.6	Potato
Conveyor	1.5	4.6×10^6	11.3	Cod cakes
Steam clean room	16	$\geq 1.4 \times 10^7$	20.2	-
Wall in rack washing area	6	-	-	Poultry products
Inspection belt guard	48	-	-	Peas

METHODS

Strains

The strains used for this work were *Pseudomonas aeruginosa* (NCIB 10421) and *Staphylococcus aureus* (NCTC 10788).

Maintenance of Cultures

For long term storage, the bacteria were kept on Cryobeads (Lab M) at -18°C. Once per week a bead was added to 150ml Nutrient Broth (Oxoid) and shaken at 30°C for 16hr. This master culture was then used to inoculate further cultures for up to one week.

Culture of Organisms

Overnight cultures were routinely used. These were obtained by inoculating 150ml Nutrient Broth with 1ml of master culture and shaking at 30°C for 16hr.

Swabbing

Surfaces were swabbed with cotton swabs (Sterilin) pre-moistened with diluent (20ml) (see Appendix 1), and placed in a 10ml volume consisting of 9ml diluent and 1ml inactivator (see Appendix I). The organisms present were resuspended from the swab by vortexing for 30 seconds. The resuspension fluid was serially diluted in diluent for total viable count determination.

Total Viable Count (TVC) Determination

The resuspension fluid (from the swab) was serially diluted in diluent and duplicate 1ml samples were removed for pour plating using Nutrient Agar (Oxoid). Plates were incubated at 30°C for 2 days. Counts were calculated as cfu/cm^2, and the log reductions calculated by subtracting the log count (cfu/cm^2) of treated surfaces from the log count of control untreated surfaces.

Direct Epifluorescent Microscopy (DEM)

Surfaces were stained with DEFT buffered acridine orange (Difco) for 2 min, washed gently in distilled water, drained and left to air dry (Holah *et al*, 1989). Samples were examined at 1000x magnification and enumerated using an epifluorescence microscope linked via a video camera to an Optimax V image analyser (Synoptics Ltd., Cambridge). Orange fluorescing bacteria in 20 fields of view were counted and counts were expressed as percentage area coverage. Percentage area covered was converted to counts/cm^2 using a calibration based on measuring the size of twenty individual organisms. The typical sizes of *P.aeruginosa* and *S.aureus* were 1.45 and 0.61μm^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.

The DEM count is a total count of both viable and non viable cells present on the surface. The log reductions were calculated by subtracting the log count (cells/cm^2) from treated surfaces from the log count from control untreated surfaces. The log reductions determined from DEM counts reflect removal of cells from the surface, whilst the TVC log reductions are influenced by loss in viability of the attached population.

Biofilm Development

Monolayer Biofilm Development

Suspensions of either *P.aeruginosa* or *S.aureus* were prepared by centrifugation of overnight cultures at 3600g for 10 min. The pellets were resuspended in 0.1M phosphate buffer adjusted to pH 7.0 with NaOH. Stainless steel coupons (100 x 40mm) with a 2B finish were washed in detergent and sterilised by autoclaving at 121°C for 15 min. Bacterial attachment was initiated by adding the suspension to the coupons, and incubating at 25°C. After 1hr the bacterial suspension was removed and replaced with growth medium (Appendix I) for 4hr at 25°C. After this period an incomplete monolayer of either *S.aureus* or *P.aeruginosa* had developed on the surface.

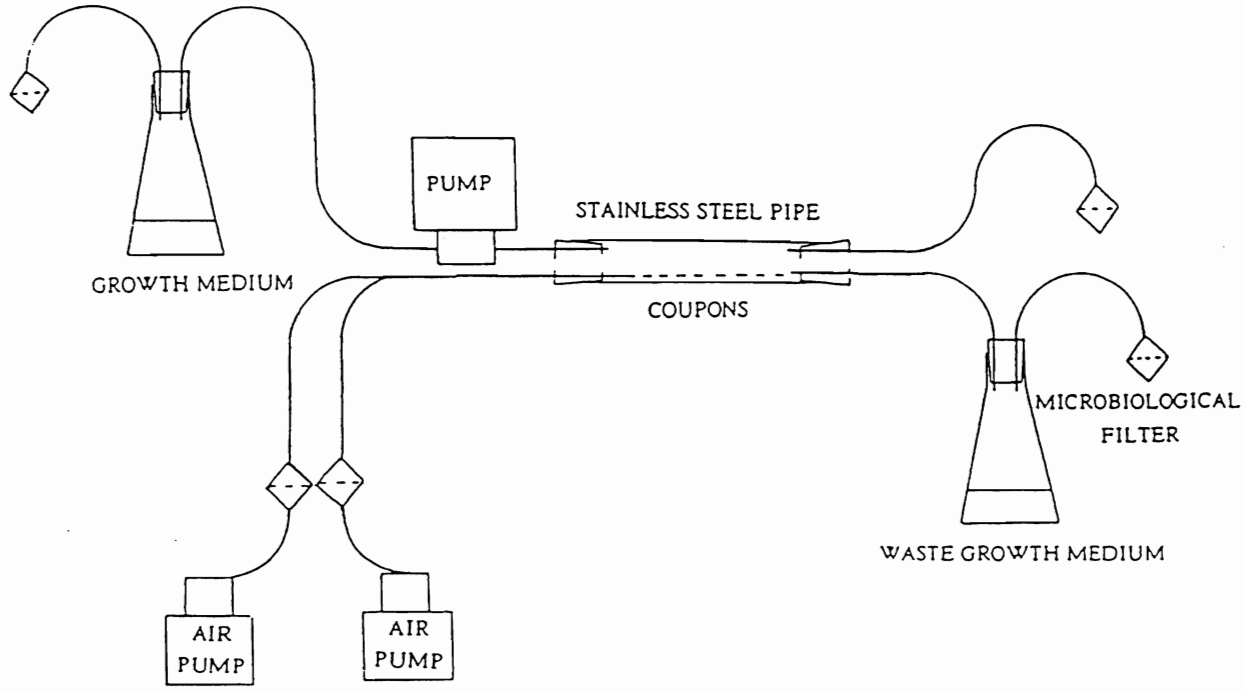
Multilayer Biofilm Development

Figure 1 shows a schematic representation of the apparatus used to develop the biofilms. The apparatus was designed to provide a continuous flow of fresh growth medium to the coupons with attached bacteria and a supply of filtered air to provide oxygenation.

Bacterial attachment to stainless steel coupons (10 x 10mm) was initiated by adding bacterial suspension in buffer to the coupons in a Petri dish. After 1hr the coupons were removed and placed in the assembled sterilised apparatus for up to 24hr. The growth medium was fed in to the pipe through silicone tubing passing through a rubber bung at one end of the pipe (entry end) from a reservoir by a positive displacement peristaltic pump (Watson and Marlow 502s at flow setting 5). The level of growth medium (to just cover the coupons) on the pipe was set by the position of the overflow tubing through the bung at the exit end of the pipe which led to a waste container. Filtered air (0.2μm pore size, Acrocap, Gelman Sciences) was supplied from two small fish tank pumps, fitted through the entry bung, positioned to provide air flow into the growth medium. Equilibration of air pressure within the pipe was maintained by a filtered air outlet on the exit bung.

After the 24hr growth period, the flow of growth medium was suspended, the pipe emptied and the coupons removed. The effects were examined of different detergents alone and in conjunction with disinfectants on the viability of these multilayers.

Figure 1 - Multilayer Biofilm Development Apparatus



Cleaning Studies

Detergents Used

Generic Type	Trade Name	Manufacturer	Recommended conc. % (v/v)	Method of Application and Contact Time
Alkaline (pH 11.6)	Easyclean	Maigret Chemicals	5	Soak - 20 min
Acidic (pH 1.7)	Ambersan	Tampen + Tampen Ltd	2.5	Soak - 20 min
Neutral (pH 8.3)	SU121	Lever	1	Soak - 20 min
Gel alkaline	Shuregel No. 2	Diversey	1-5	Gel applicator - various contact times
Foam alkaline	Shurefoam No. 1	Diversey	2-5	Foam lance attachment to Kew system - various contact times

Pressure Washing

A KEW system (KEW Cleaning Systems Ltd., Penrith, Cumbria) was used to spray wash surfaces at 250, 500, 750, and 1000 psi, and in addition surfaces were sprayed using the KEW system without switching the pump on to give a pressure of 110 psi. The nozzle of the lance was routinely placed 20cm from the surface, although certain experiments examined the effect of distance on the removal of organisms. The distances examined were 6, 12.5, 25, 37.5 and 50cm between the spray nozzle and the surface. A spray time of 5 seconds was routinely used except for experiments investigating the effect of spray time at constant pressure where times of 1, 3, 5, 7 and 10 seconds were examined.

Mains water at ambient temperature was used for all experiments except those examining the effect of water temperature on the removal of attached microbes. For this work the KEW spray rig was connected to a reservoir tank containing water at the temperature under test (25, 35, 45 or 55°C).

CIP Cleaning

The effects of cleaning velocity (0.25, 1.0, 1.25, 1.5, 2.25, and 3.0m/s) for 20 min and cleaning times (5, 10, 20, 45, 60 and 90 min) at 1.5m/s were investigated on the removal of attached bacteria. Monolayers of *P.aeruginosa* were grown on stainless steel stubs (1cm in diameter) as described previously. These stubs were inserted in the sample holder so that the stub surface was flush with the interior surface of the pipe.

The sample holder was then connected to a simple CIP loop and cleaned at a particular flow rate for a certain time using an alkaline detergent (Holah *et al*, 1992) at 60°C. After certain time intervals, stubs were removed, rinsed gently in phosphate buffer to restore an appropriate pH and the remaining attached population enumerated by swabbing and TVC or DEM as described previously.

Steam Cleaning

The effectiveness of a steam cleaner (Raporella, Interselect by Polti Ltd., Trowbridge, Wiltshire) in terms of removal and reduction in the viability of attached populations was evaluated using monolayer biofilms of *P.aeruginosa* and *S.aureus*.

Disinfection Studies

Surface Disinfectant Test

Stainless steel discs (10mm diameter) with a 2B finish were washed in mild detergent, rinsed and sterilised by autoclaving at 121°C for 20 minutes. Biofilms of either *S.aureus* or *P.aeruginosa* were grown as described above.

Discs with attached biofilms were placed individually in 4ml 0.03% (w/v) bovine albumin solution. After 2 minutes, 4ml of double strength disinfectant solution made up with water of standard hardness (WSH) was added. After 5 minutes contact time the discs were transferred to 9ml inactivation liquid for 5 minutes.

After inactivation each disc was transferred to a Malthus tube containing 4ml SPYE and incubated at 30°C in a Malthus 2000 growth analyser (Malthus Instruments, UK) until a change in conductance occurred and a detection time was obtained. The Malthus 2000 growth analyser monitors microbial growth by measuring changes in the electrical conductance of the culture media. The time taken to produce a measurable change in conductance is inversely proportional to the number of organisms present initially.

Effect of Detergent on the Efficacy of Disinfection

Monolayer biofilms

Duplicate discs with attached biofilms of *S.aureus* or *P.aeruginosa* were immersed in detergents for a range of specified contact times (1, 60, 120, 180, 240 and 300 seconds). One of the discs was then gently washed in a Petri dish of water and placed in a Malthus tube containing 4ml SPYE. The other disc was placed in 0.03% (w/v) bovine albumin for 2 minutes and the surface disinfectant test procedure followed.

Biofilm controls were prepared using 4ml WSH instead of disinfectant.

Four detergents, each made up using WSH to the maximum concentration recommended by the manufacturer, were assessed. The four detergents used were:

i) A foam cleaning strongly alkaline detergent at 5% (v/v) (pH 12.7 approx).

ii) A low alkaline spray wash detergent at 3g/litre (pH 11.6 approx).

iii) A neutral general purpose detergent at 6mg/litre (pH 8.3 approx).

iv) A foam cleaning acidic detergent at 5%(v/v) (pH 1.7 approx).

Multilayer biofilms

The effect of detergent on the efficacy of disinfection against multilayer biofilms was examined by growing 24hr multilayer biofilms as described above. Triplicate surfaces with attached biofilms of either *S.aureus* or *P.aeruginosa* were immersed in detergent or diluent (as untreated control) for 20 minutes. Surfaces were then transferred to inactivator for 5 minutes. The effect of detergent was determined by vortexing the surfaces for 30 seconds to remove the attached cells and serial dilution for TVC analysis. The added effect of disinfectant (either 1% quaternary ammonium compound or 1% biguanide) was determined by transferring triplicate surfaces to disinfectant solution or diluent for 5 minutes, and then transferring the surfaces to inactivator for 5 minutes before vortexing, dilution and TVC analysis (section 2.5).

Effect of Temperature on the Efficacy of Disinfection

Bovine albumin, inactivator and disinfectant were held at 4°C, 22°C, 37°C or 58°C for a minimum of 30 minutes to reach equilibrium. Discs with attached biofilms were then added to the 0.03% (w/v) bovine albumin and after a further 30 minutes incubation, to allow the discs to reach the desired temperature, 4ml of double strength disinfectant was added. After 5 minutes exposure the discs were transferred to 9ml inactivation liquid for 5 minutes.

Biofilm control discs were prepared for each temperature by placing in 0.03% (w/v) bovine albumin and incubating at the appropriate temperature for 30 minutes with WSH added instead of disinfectant.

Discs were then transferred to Malthus tubes containing 4ml SPYE and incubated at 30°C until a change in conductance occurred and detection times were obtained.

Examination of the Effect of Temperature and Humidity on Attached Microbes

Biofilms of *S.aureus* and *P.aeruginosa* grown on stainless steel coupons were placed in desiccation chambers containing the saturated solutions detailed below to give a range of different humidities.

Saturated solution	Relative humidity (%) at particular temperatures			
	10°C	25°C	37°C	58°C
KOH	12	8	6	5
$MgCl_2$	34	33	32	30
NaCl	76	75	75	75
Distilled water	100	100	100	100

In addition, an extractor system of a particular food processing site, which was found to generate substantial biofilms, and had multiple sites for attachment of stainless steel plates, was used to generate biofilms to place in the humidity chambers described above at 10, 25, 37 and 58°C.

The coupons were sampled at various time intervals by TVC or DEM.

Scanning Electron Microscopy (SEM)

Stainless steel coupons (10mm x 10mm) with attached biofilm (either monolayer or multilayer) were fixed in 2.5% (v/v) glutaraldehyde in 0.1M phosphate buffer (pH 7.0) for 10 hr, then air dried. The coupons were then mounted on SEM stubs with double sided tape, and the conductivity between the coupon and stub ensured by the application of electro DA6 915 high conductivity paint (Achesom Colloids Co., Plymouth). The coupons were then gold sputtered and examined with a Cambridge 56000 scanning electron microscope.

Replication and Statistical Analysis

In most of the biofilm experiments triplicate stainless steel coupons were used for each treatment, and the experiments were performed on three separate occasions. In certain cases, where there was more variability, five replicate samples per treatment were used. This was necessary for the studies examining the efficiency of the different cleaning techniques against the naturally occurring factory biofilms.

Differences in the number of bacteria remaining on the surfaces after each particular treatment were assessed statistically by analysis of variance (ANOVA); significance is expressed throughout this work at the 95% confidence level or greater.

RESULTS AND DISCUSSION

In order to examine various cleaning parameters, monolayer and multilayer biofilms were developed on stainless steel coupons. Studies of surface populations in the factory environment showed that biofilms tended to comprise collections of microcolonies (Gibson *et al*, 1995) and therefore the biofilms developed in the laboratory were similar to those found in factory environments. Multilayer biofilms were occasionally found in the factory environment, and therefore multilayer biofilms were developed in the laboratory for the study of detergent and disinfection interactions. Previous studies in the factory environment showed that *Pseudomonas* and *Staphylococcus* species were particularly common on the surfaces, and for this reason *P.aeruginosa* and *S.aureus* biofilms were developed for use in the cleaning studies. In addition these two species represent Gram positive and Gram negative bacterial groups.

Influence of spray water pressure and temperature on the removal and viability of biofilms

Figure 2 shows the log reductions achieved by high pressure water spraying on *P.aeruginosa* and *S.aureus* biofilms. Statistical analysis of the results shows that spraying with water at pressures above 250 psi did not significantly increase the removal of either *S.aureus* or *P.aeruginosa* biofilms. Previous work has demonstrated that the aerosol generation from high pressure systems may cover an extensive area (Holah *et al* 1990b, 1993a). The usage of 250psi as opposed to higher pressures may limit to some extent the potential spread of contamination due to aerosols. However, cleaning programmes are generally designed for product residue removal, rather than biofilm removal and therefore higher pressures may be required for the removal of some soils.

Figure 3 shows the log reductions achieved by high pressure water spraying on dried on *P.aeruginosa* and *S.aureus* biofilms. Similar trends were observed, i.e. no further increase in removal was achieved by increasing the spray pressure. However, the log reductions achieved were lower for dried-on biofilms, demonstrating that these dried on organisms were more difficult to remove. The lower log reductions observed in the TVC measurements reflect the loss of viability of the surface microorganisms through drying.

Figures 4 to 7 show the effect of water temperature on the removal of *P.aeruginosa* biofilms as measured by DEM and TVC. Figures 5 and 8 show the log reductions produced as measured by swabbing and TVC, whilst Figures 6 and 7 show the data obtained by DEM. The results show that increasing water temperature did not significantly increase the removal of *P.aeruginosa* biofilms, but a temperature of 55°C reduced the viability of *P.aeruginosa* (Figure 8).

Figures 8 to 11 show the effect of the water temperature on the removal and viability of *S.aureus* biofilms. Similar trends were observed; increasing water temperature did not increase the removal of *S.aureus*, but increasing water temperatures did reduce the viability of *S.aureus*. The practical implications of these data are that the use of hot water (55°C) does not improve the removal of bacterial biofilms, although using hot water does reduce the viability of attached microbes and potentially those released from the surface in aerosols. In addition, hot water may be required to achieve effective soil removal, particularly in the case of fatty soils.

Figure 2 Effect of high pressure / low volume cleaning on the removal of *P.aeruginosa* (P.a) and *S.aureus* (S.a) biofilms as measured by DEM and TVC

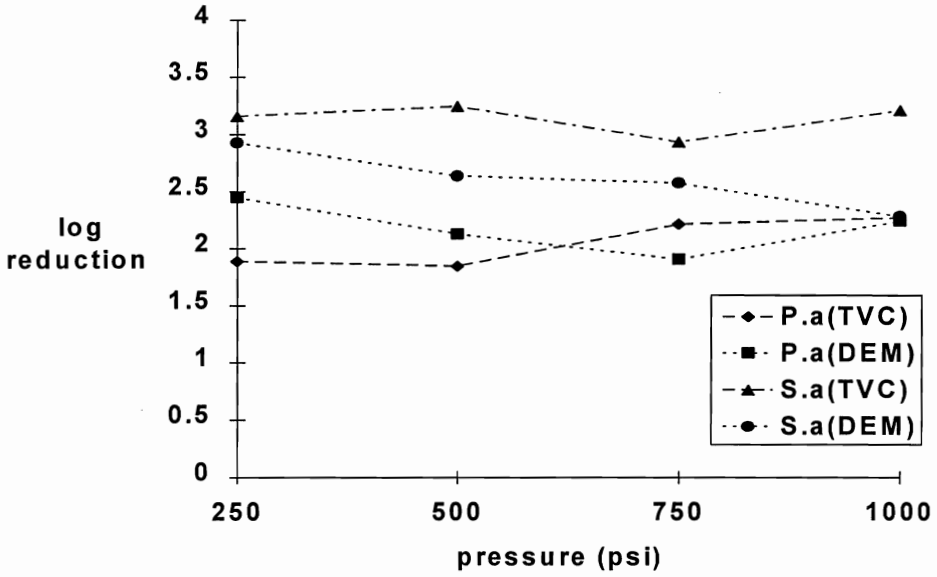


Figure 3 Effect of high pressure / low volume cleaning on the removal of dried on *P.aeruginosa* and *S.aureus* biofilms

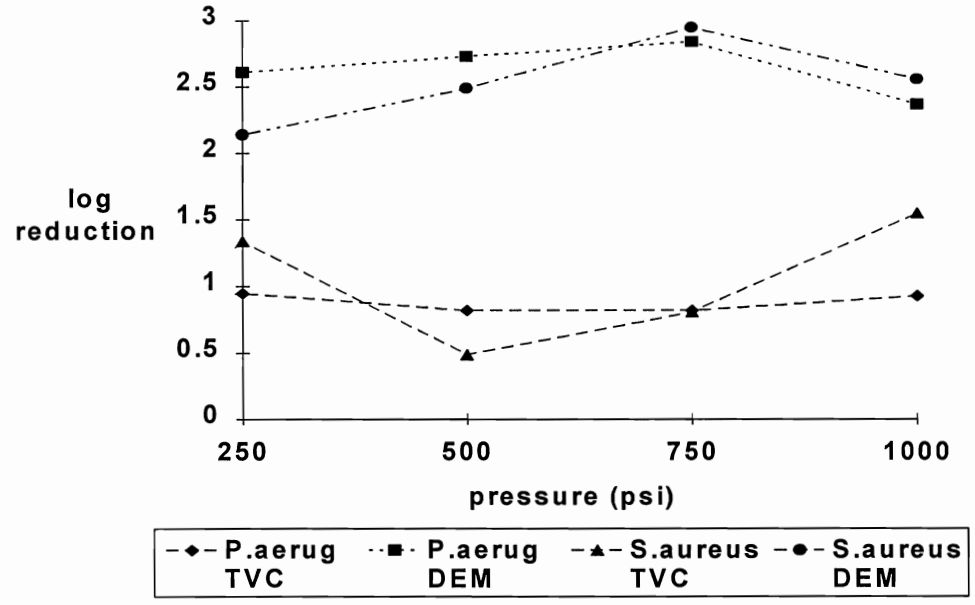


Figure 4 Effect of temperature on high pressure removal of *P.aeruginosa* biofilms as measured by TVC

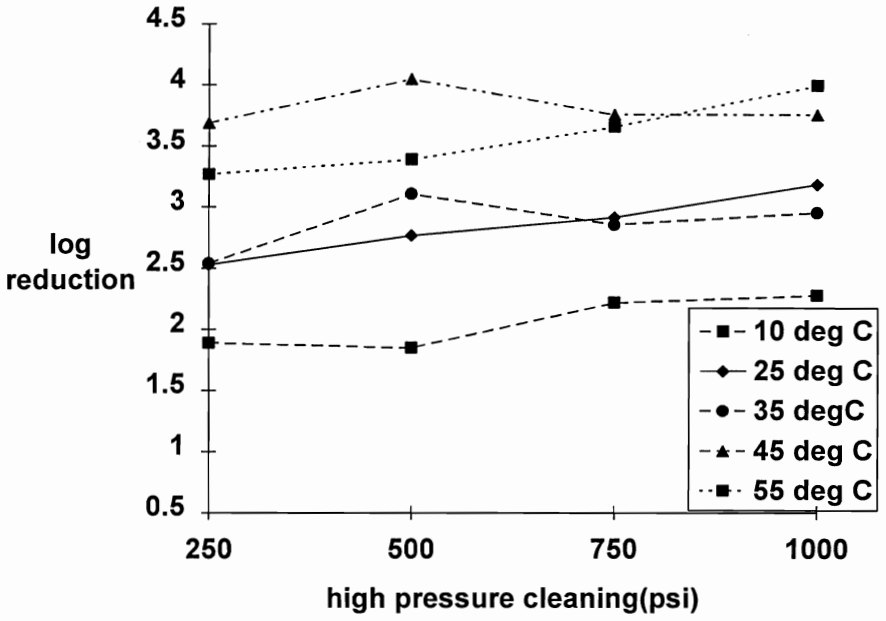


Figure 5 Effect of temperature on high pressure removal of *P.aeruginosa* biofilms as measured by DEM

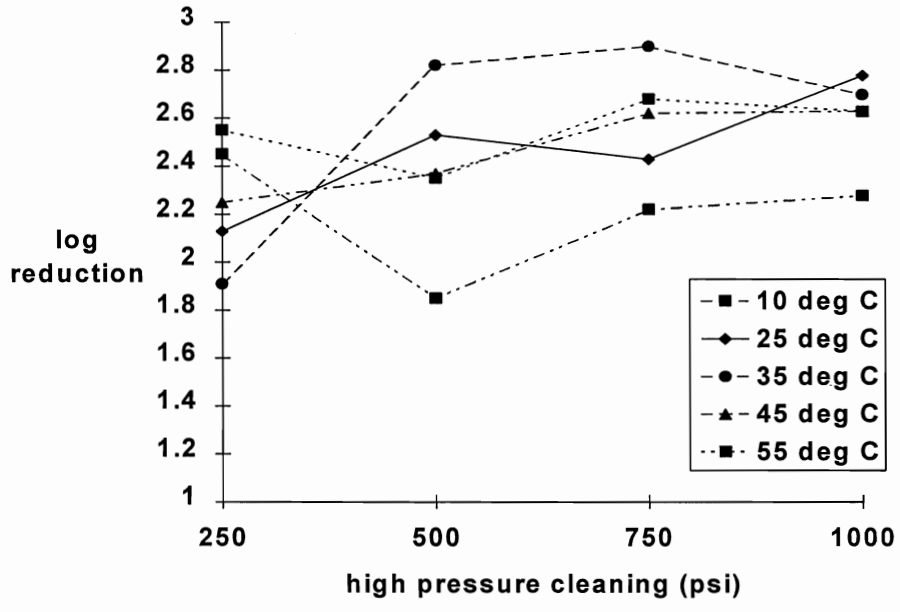


Figure 6 Effect of temperature on removal of *P.aeruginosa* biofilms at different pressures as measured by DEM.

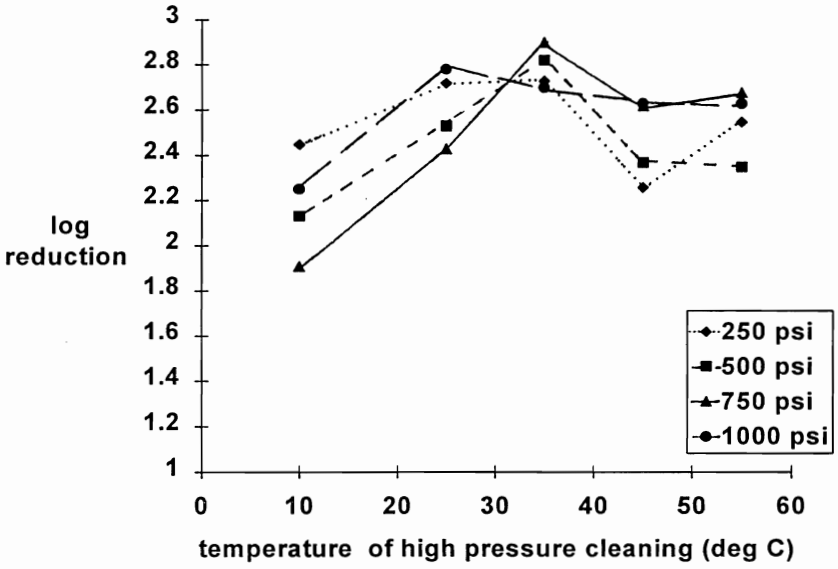


Figure 7 Effect of temperature on the removal of *P.aeruginosa* biofilms at different pressures as measured by TVC

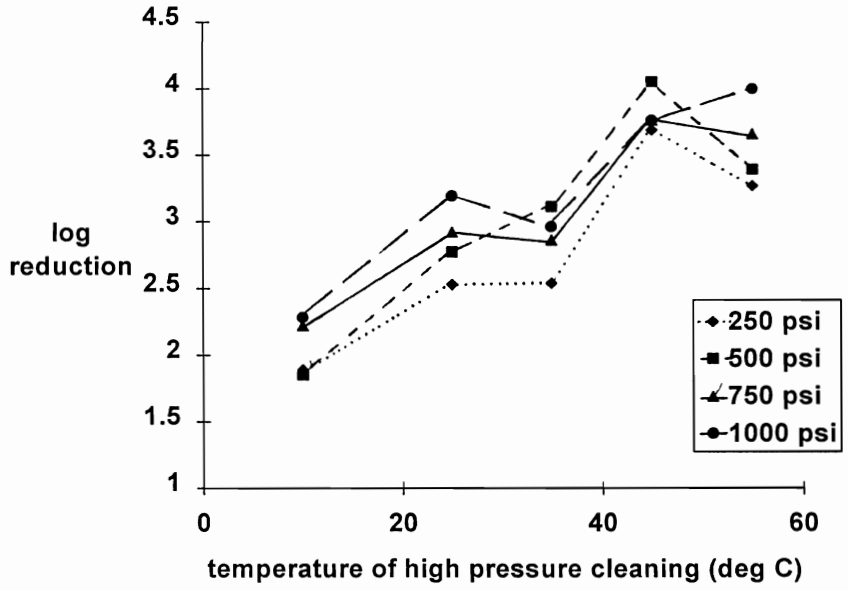


Figure 8 Effect of temperature on high pressure removal of *S.aureus* biofilms as measured by TVC

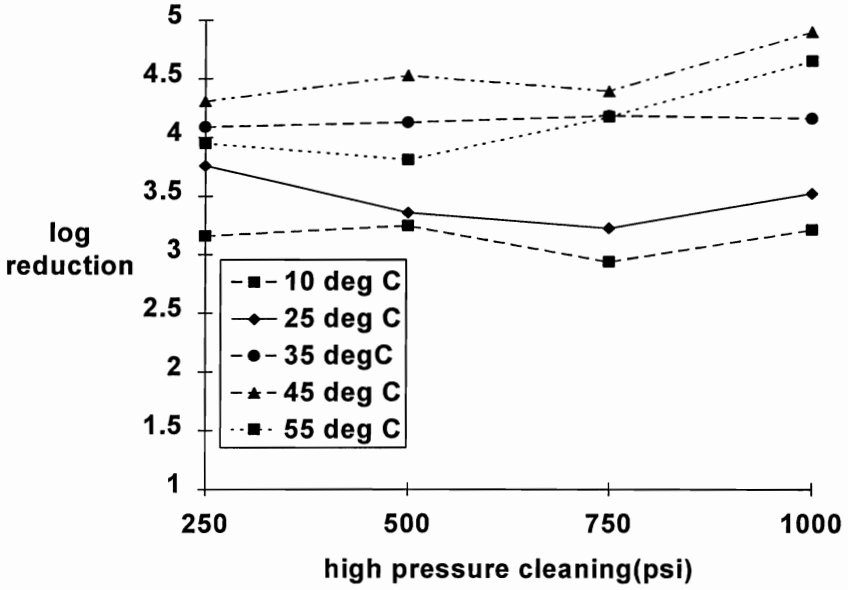


Figure 9 Effect of temperature on the high pressure removal of *S.aureus* biofilms as measured by DEM

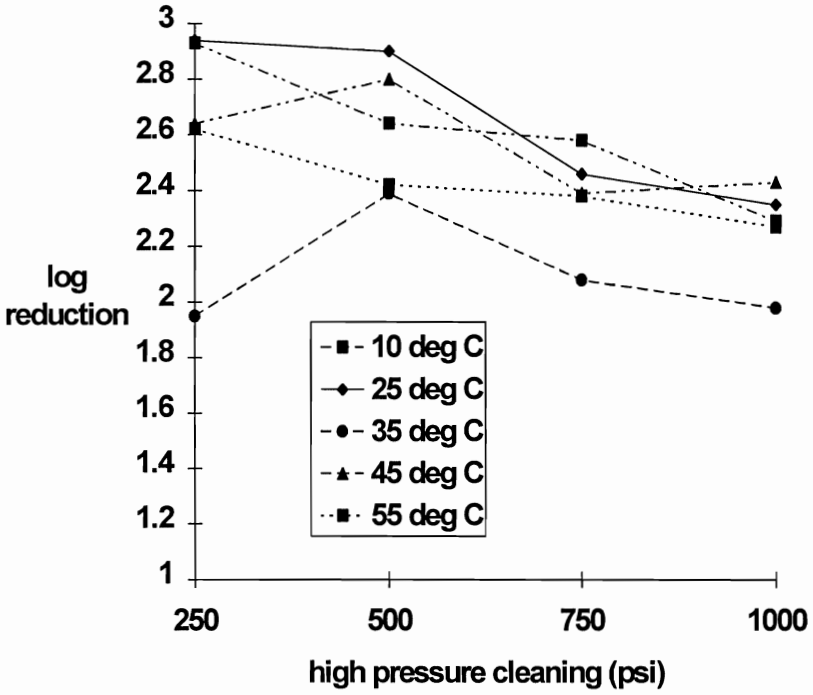


Figure 10 Effect of temperature on the removal of *S.aureus* biofilm at different pressures as measured by TVC

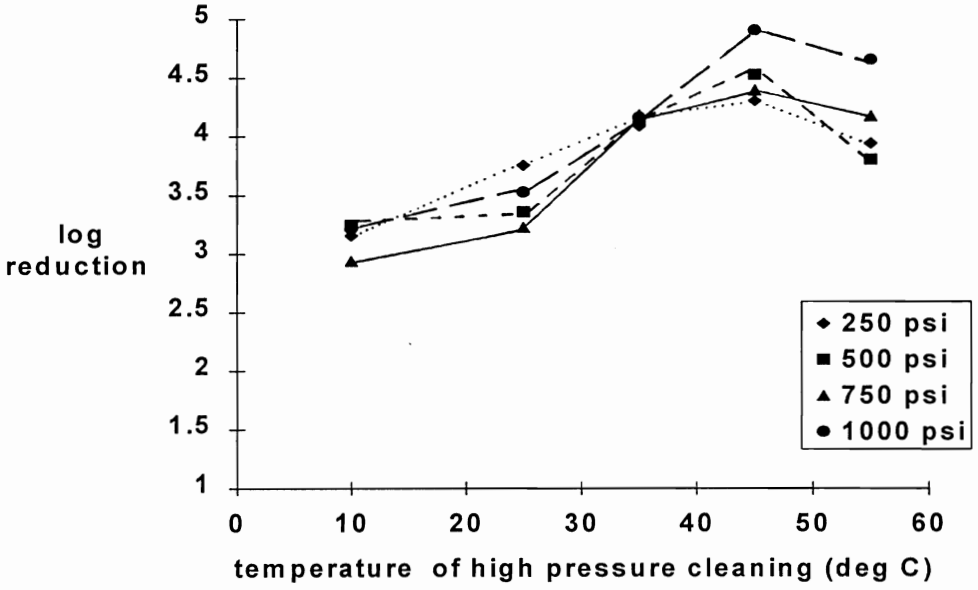
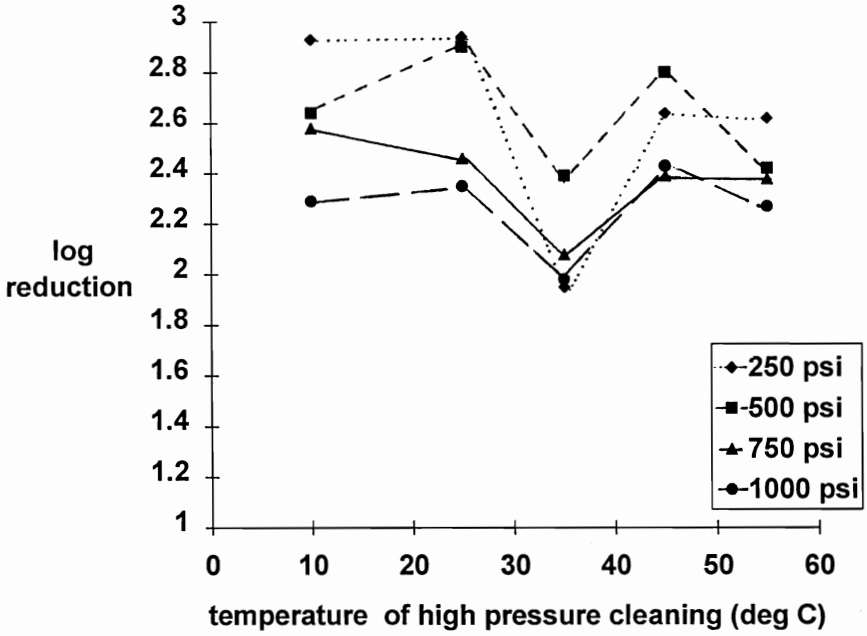


Figure 11 Effect of temperature on the removal of *S.aureus* biofilms at different pressures as measured by DEM



Effect of the distance of the spray lance from the surface on the removal of bacterial biofilms

Figure 12 shows the effect of distance of the spray lance from the surface on the removal of *P.aeruginosa* biofilms. The results show that the optimum distance for removal is between 125 and 250mm as measured by DEM. Statistical analysis of the TVC results showed that the optimum distance for removal was 250mm. The reason for the difference in removal at different distances may be due to the droplet size impacting on the biofilm. The log reductions measured by DEM and TVC at distances over 60mm were significantly different, suggesting that beyond 60mm, surface forces were such that the viability of the attached *P.aeruginosa* was significantly reduced.

Figure 13 shows the effect of distance of the spray lance from the surface on the removal of *S.aureus* biofilms. The log reductions observed were lower than those for *P.aeruginosa* although not significantly different. Analysis of the results showed that there was no significant difference in spraying at distances above 60mm at the 5% level of significance; however, spraying at 125mm produced significantly higher log reductions at the 0.1 significance level. The data would therefore suggest that the optimum distance for the spray lance from the surface is 125mm or above. In contrast to *P.aeruginosa* there was no difference between the TVC and DEM data from *S.aureus* and therefore no loss in viability of attached *S.aureus* In summary, the maximum amount of removal of *P.aeruginosa* and *S.aureus* biofilm was achieved when the spray lance was 250mm from the surface.

Figure 12 Effect of distance of the spray lance from the surface on the removal of *P.aeruginosa* biofilms (Pressure 250 psi)

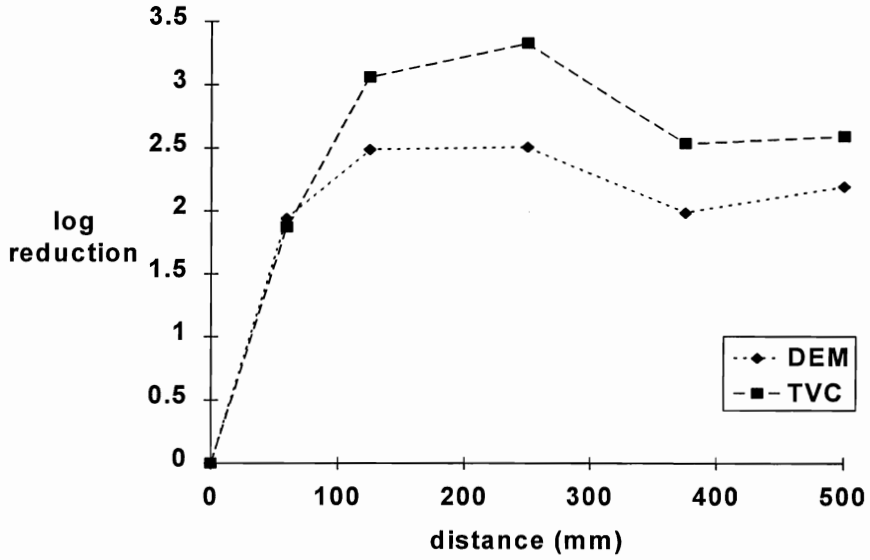
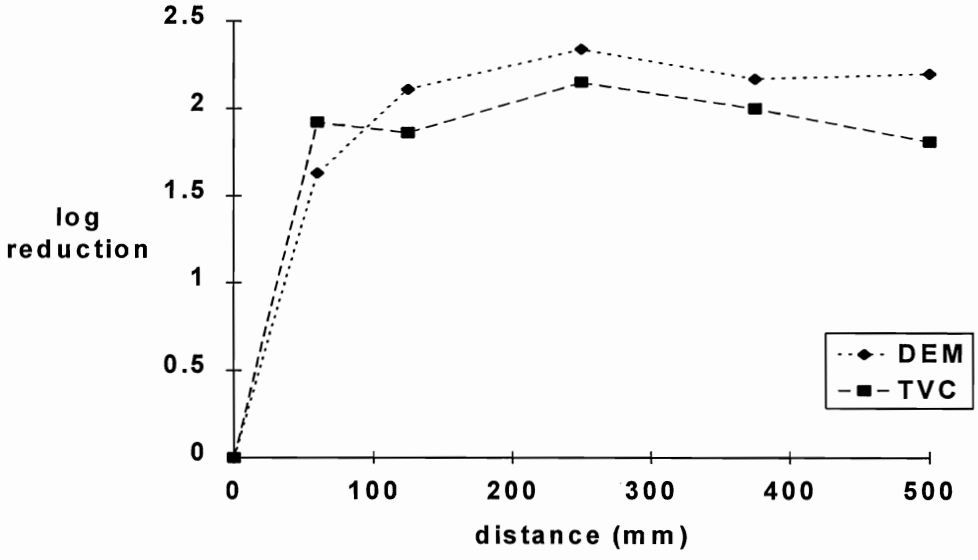


Figure 13 Effect of distance of the spray lance from the surface on the removal of *S.aureus* biofims (Pressure 250 psi)



Effect of high pressure spray cleaning time on the removal and viability of biofilms

Figures 14 and 15 show the effect of high pressure spray cleaning time on the removal of *P.aeruginosa* and *S.aureus* biofilms respectively. In both cases, cleaning for times above 1 second did not significantly increase the removal of these organisms. The degrees of removal of *S.aureus* and *P.aeruginosa* were similar with log reductions of 2-2.5. In addition there was no significant difference between the DEM results and TVC results.

Figure 16 shows the effect of cleaning time on the removal of dried-on *P.aeruginosa* biofilms. The DEM data shows that spraying dried biofilms for greater than one second up to ten seconds did not significantly increase the removal of dried-on *P.aeruginosa.* The DEM count is a total count and therefore reflects both viable and non viable cells present on the surface and as such DEM measures cell removal. The TVC results demonstrate that drying had a significant effect on the viability of *P.aeruginosa.* The control unsprayed biofilms gave DEM counts of 10^7 cells/cm² approximately, whilst the TVC results showed 10^5 cfu/cm² approximately and therefore two log orders were lost due to desiccation. High pressure spraying of the surfaces produced a two log reduction in the DEM count; however, the log reduction as measured by TVC was close to zero. A slight negative log reduction was observed, which suggests that *P.aeruginosa* had increased in numbers; however, it is more likely that this is an error due to swabbing a surface with low numbers of cells, or to enhanced recovery after spraying. The relationship between DEM and TVC is generally good (as shown by the previous graphs); however, when low numbers are present on the surface the problems of recovery by swabbing are more marked. In contrast Figure 17 shows that the TVC results for the more desiccation-resistant *S.aureus* show similar trends to those observed for DEM as no significant increase in the removal was observed with spray times greater than one second.

In the factory, times of 1 second are likely to be typical. These results show that spraying for longer than one second does not increase the removal of bacterial biofilms.

Figure 14 Effect of high pressure (250psi) cleaning time on the removal of *P.aeruginosa* biofilms

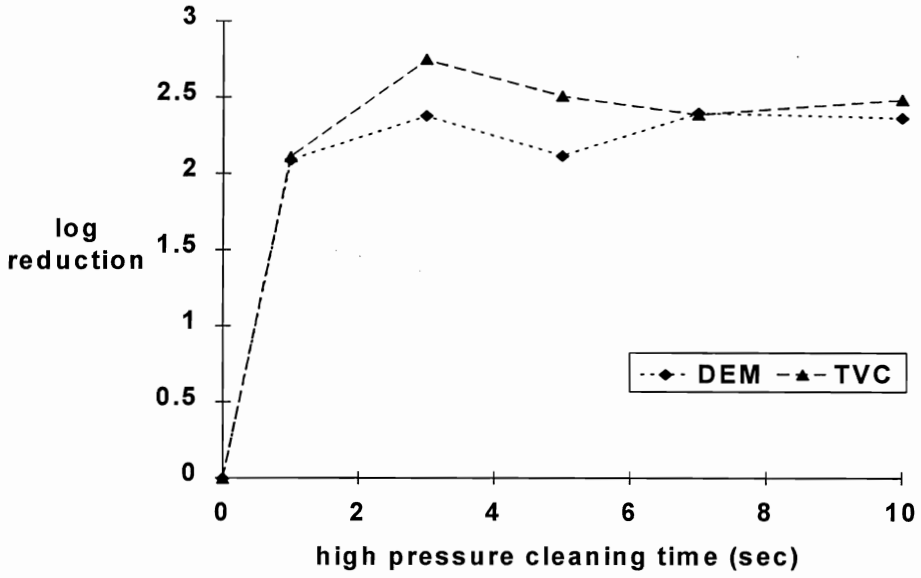


Figure 15 Effect of high pressure (250psi) cleaning time on the removal of *S.aureus* biofims

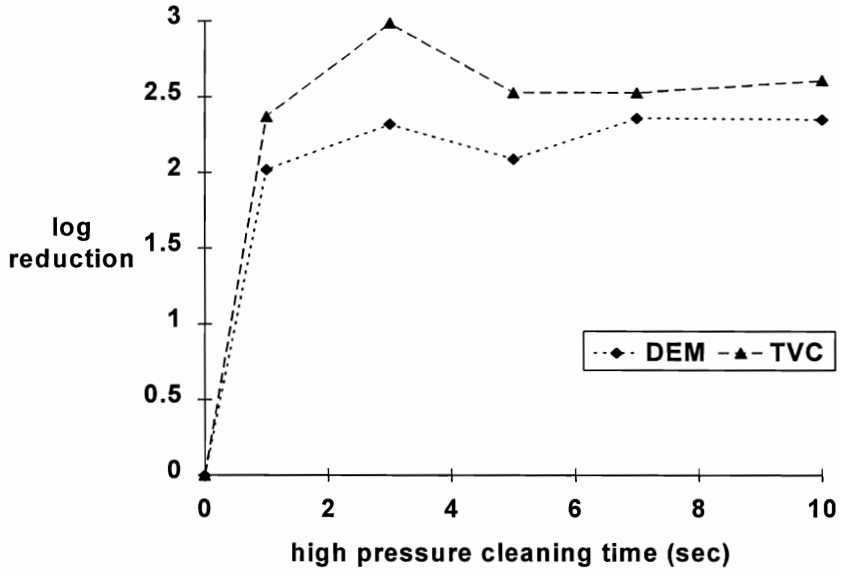


Figure 16 Effect of high pressure (250psi) cleaning time on the removal of dried on *P.aeruginosa* biofilm

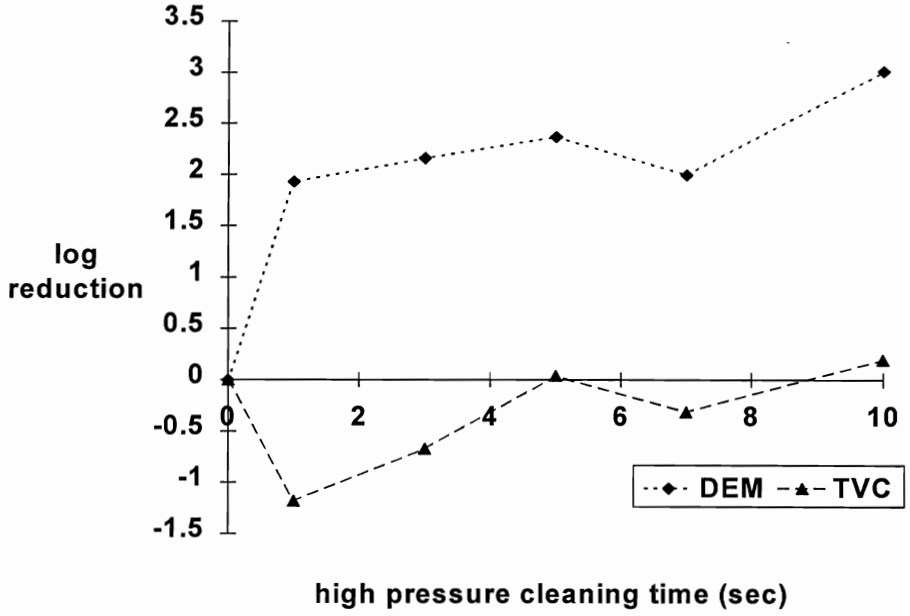
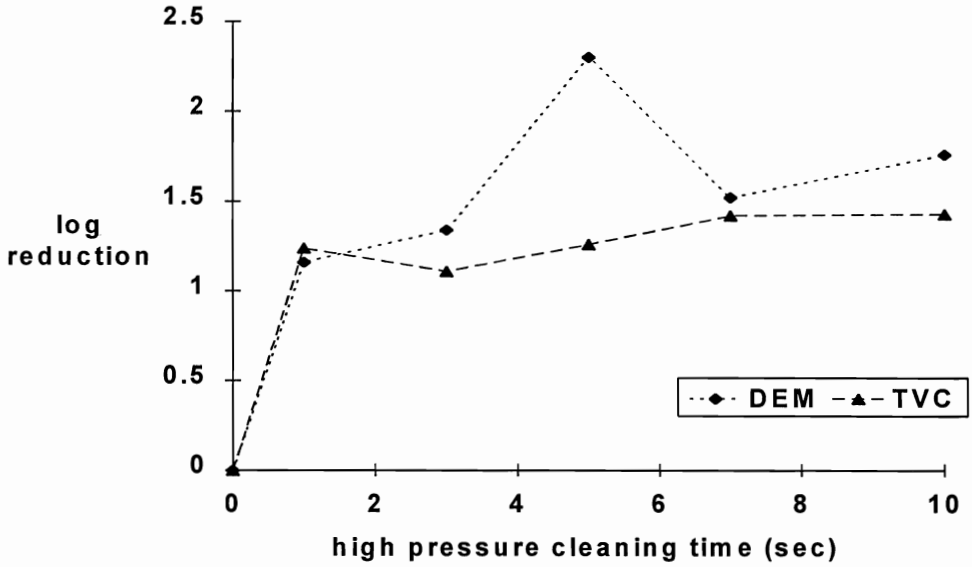


Figure 17 Effect of high pressure (250psi) cleaning time on the removal of dried on *S.aureus* biofilm



Effect of gel or foam contact times on the removal and viability of biofilms

Figures 18 and 19 show the effect of gel and foam contact times on the removal of *P.aeruginosa* biofilms. Biofilms were exposed to the detergent for the specified times, and then rinsed gently in distilled water before enumeration by DEM or TVC. The gel and foam products were similar in that both were alkaline detergents (pH 12 and 11.7 respectively) and both contained disinfectant although the disinfectant in the gel was a quaternary ammonium compound and that in the foam was hypochlorite. Stainless steel plates with attached biofilms were treated whilst in a horizontal position to allow investigation of longer contact times. Figure 18 shows that under the experimental conditions (i.e. a gentle rinse) low log reductions were observed. This means that the removal of *P.aeruginosa* from the surface was low, and no significant difference was observed in the removal with gel contact time (DEM results). In contrast, log reductions in excess of three log orders were observed when the surface population was enumerated by Total Viable Count analysis. The fact that there was relatively little removal, yet high log reductions detected in the TVC data shows that the gel significantly reduced the viability of the attached *P.aeruginosa.* At contact times between 1 and 20 minutes there was no significant increase in loss in viability with contact time; however, contact times of 30 and 40 minutes produced significantly higher log reductions (5% significance level).

Figure 19 shows the effect of foam contact time on the removal and viability of *P.aeruginosa.* In this case the combination of foam and a gentle rinse resulted in approximately a 2-2.5 log reduction, which is not significantly affected by the foam contact time. Comparison of the log reductions achieved for *P.aeruginosa* using gel and foam shows that the foam detergent treatment resulted in greater removal of attached *P.aeruginosa.* The TVC data demonstrates that the foam had a significant effect on the viability of *P.aeruginosa* biofilms, producing a four log reduction in the attached population. The TVC quantifies viable cells only, and as a certain amount of removal was detected by the DEM results, the high log reductions observed are the net result of removal of cells, and reduction in the viability of the attached population. Increasing the contact time did not significantly increase the log reductions determined by TVC.

Figures 20 and 21 show the effect of gel and foam respectively on *S.aureus* biofilms. Figure 20 shows that (as was observed for *P.aeruginosa*) the removal of the biofilm by gel was relatively ineffective, resulting in approximately one log reduction. Increasing the gel contact time did not significantly increase this removal. The TVC data demonstrates that the foam significantly affected the viability of the attached *S.aureus*, producing overall log reductions (i.e. removal and loss of viability) of approximately six log orders.

Figure 21 shows that in comparison to the gel, the foam produced greater log reductions, as measured by DEM, and was therefore more effective in the removal of *S.aureus* biofilms. This was similar to the observations for *P.aeruginosa.* The log reductions determined by TVC analysis were approximately 2.5-3 for all contact times tested, and therefore the foam chemical, in addition to removing *S.aureus,* also significantly affected the viability of *S.aureus.* Comparison of the *P.aeruginosa* and *S.aureus* results showed that the gel detergent was more effective in terms of the reduction of *S.aureus* cell viability (and therefore the QAC was more effective than the hypochlorite), whilst the gel and foam detergents showed similar effects on the viability of *P.aeruginosa* biofilms. In terms of the removal of biofilms the foam product was more effective.

Generally, detergent products are designed and selected for their effectiveness in terms of soil removal; these results show that there are differences between the biofilm removal properties of these products. Detergent contact time is important in the removal of product soil; however, the data presented here show that increasing contact times above one minute did not improve the removal of these biofilms. The fact that detergents reduce the viability of the attached population in addition to removing attached microorganisms may have important implications for the food processing environment, as fewer of the organisms released from the surfaces in aerosols, for example, will be viable and hence be a risk to the quality and safety of the product.

Figure 18 Effect of gel contact time on the removal and viability of *P.aeruginosa* biofilms

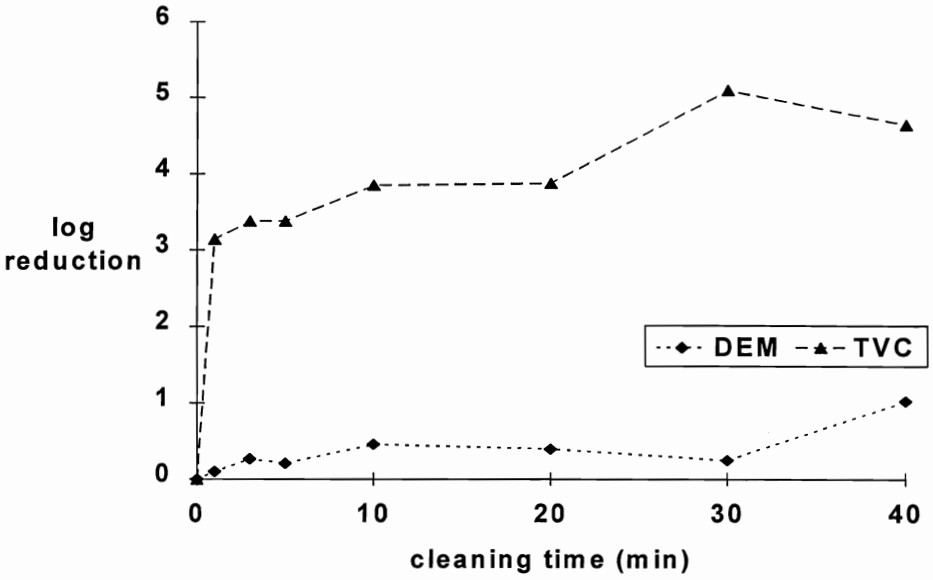


Figure 19 Effect of foam contact time on the removal and viability of *P.aeruginosa* biofilms

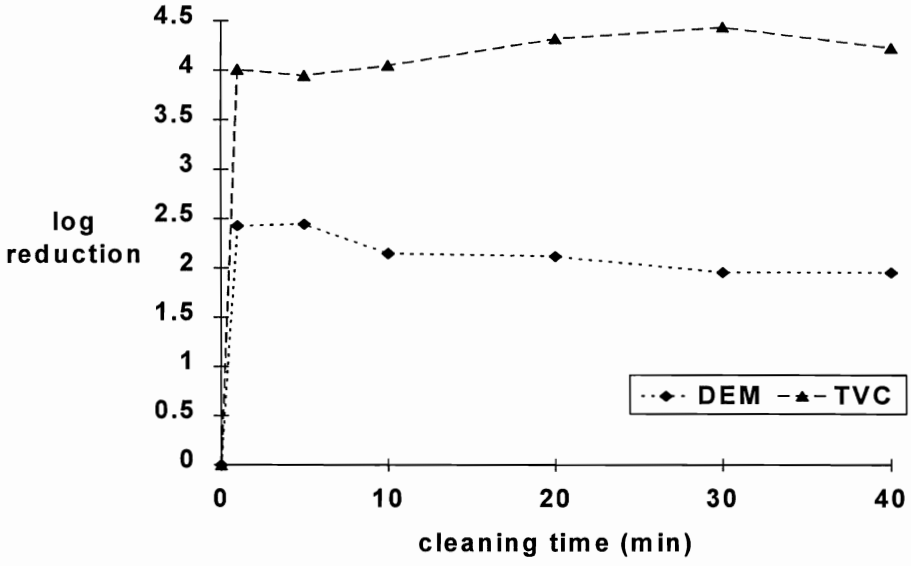


Figure 20 Effect of gel contact time on the removal and viability of *S.aureus* biofilm

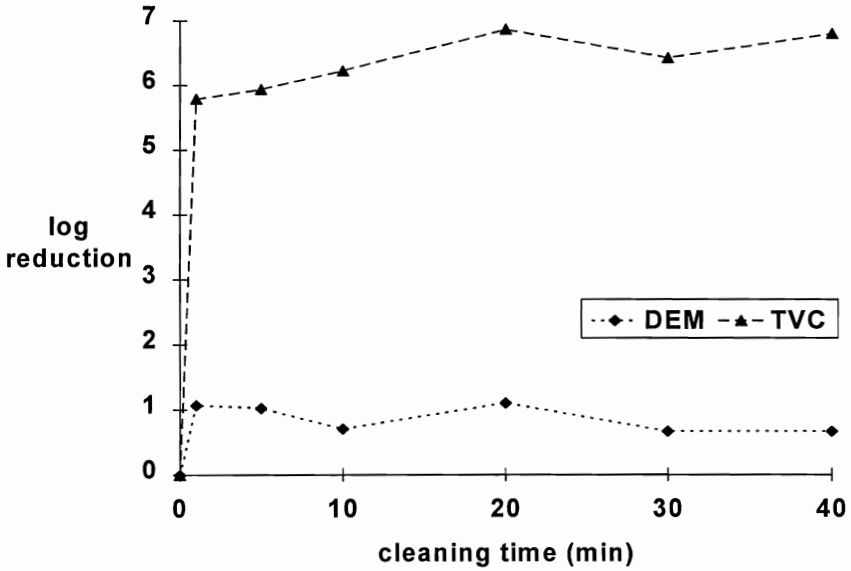
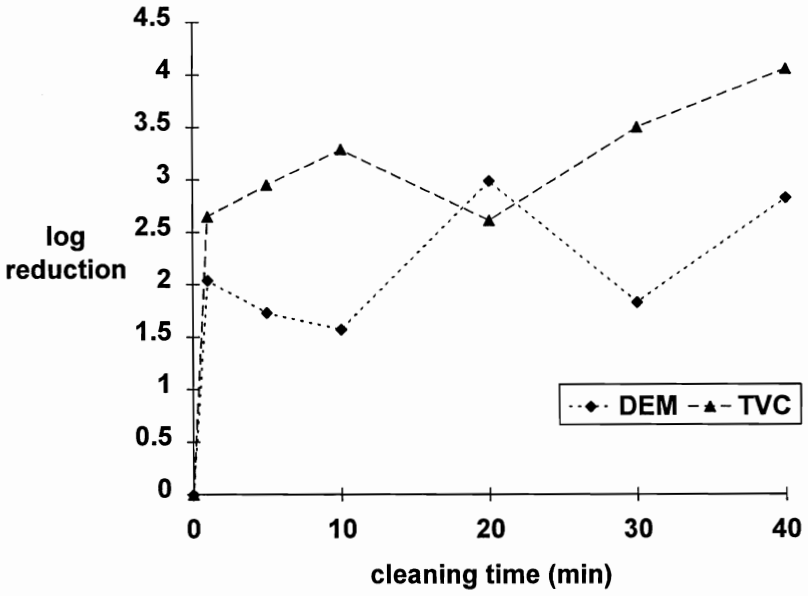


Figure 21 Effect of foam contact time on the removal and viability of *S.aureus* biofilms



Effect of different detergents on the removal and viability of biofilms

Removal of micro-organisms by detergents is quantitatively more important than disinfection in the cleaning process in terms of soil and bacterial removal as shown by Dunsmore *et al* (1981), Holah (1992) and the factory cleaning data (previous studies).

Figure 22 shows the removal of *S.aureus* biofilms using different detergents and at a range of pressures. These results show that there was no significant difference in the effectiveness of the products in terms of removal. In addition, spraying at pressures above 250psi did not significantly increase the removal of *S.aureus* biofilm. Figure 23 shows the effect of the detergents on the viability of the attached microbes. The DEM data in Figure 22 showed that approximately two log orders were removed from the surface; however, the log reductions observed in the TVC data are in excess of three log orders. This shows that the detergents reduce the viability of the attached *S.aureus.* The acidic product produced the highest log reductions, and there was no significant difference between the alkaline and neutral products.

Figure 24 shows the removal of *P.aeruginosa* biofilms using different detergents and a range of pressures. The level of removal of *P.aeruginosa* biofilms was similar to that observed for *S.aureus* at approximately three log orders. In addition, there was no significant difference in the effectiveness of the detergents in terms of removal of *P.aeruginosa.* Figure 25 shows the effect of the detergents on the viability of *P.aeruginosa.* In the case of this organism the alkaline product was most effective, although the log reductions as measured by TVC are generally lower for *P.aeruginosa* than for *S.aureus.* Although the removal of *S.aureus* was only approximately three log orders, the acidic product reduced the viability of the organisms remaining on the surface so that only one log order remained (initial levels of organisms were log 7). Consequently the cleaning process was particularly effective for *S.aureus.* In contrast, *P.aeruginosa* was more resistant to the detergent products so that a maximum four log reduction was observed and therefore in excess of three log orders remained on the surface after cleaning (the initial levels of the organism were 10^7 per cm^2). Dunsmore *et al* (1981) found that an acidic product was more effective than an alkaline product against cell viability. This trend was observed in this study for *S.aureus* but not *P.aeruginosa.* Lewis *et al* (1989) found that higher pH values resulted in greater removal of *Acinetobacter* sp.

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 forms a matrix around the cells which can protect the cells from adverse conditions. The amount and nature of the polymers produced by microorganisms varies between species (Beech *et al*, 1991; Spenceley *et al*, 1992) and this may relate to the difference in the effectiveness of the detergents. This data demonstrates the importance of choosing an appropriate and effective detergent.

Increasing the water pressure above 250 psi did not significantly improve removal of the attached microorganisms and therefore the use of high pressure washing is not recommended for the removal of microbial films, particularly with the associated increase in aerosol generation. However, cleaning programmes are generally designed for product residue removal, rather than biofilm removal, and therefore higher pressures may be required for the removal of some soils.

Figure 22 Effect of different detergents and high pressure spraying on the removal of *S.aureus* biofilms

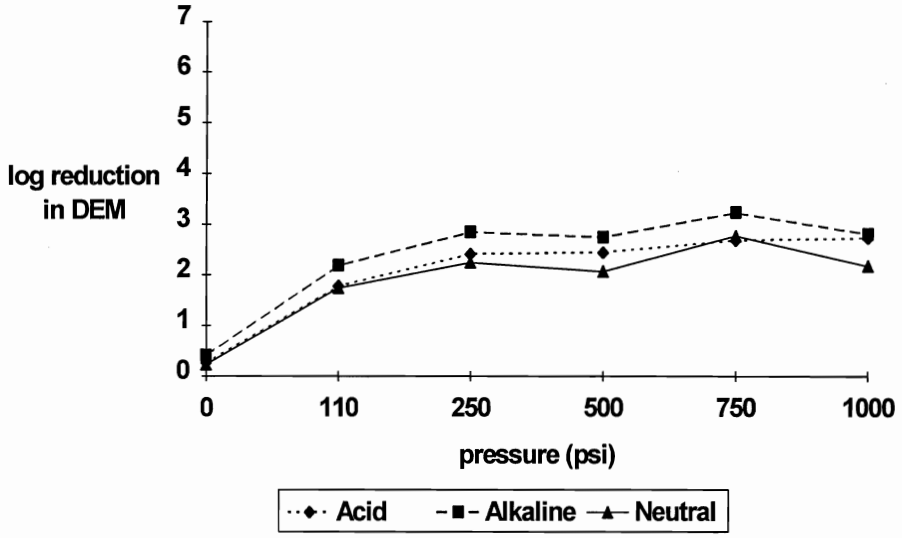


Figure 23 Effect of different detergents and high pressure spraying on the viability of *S.aureus* biofilm

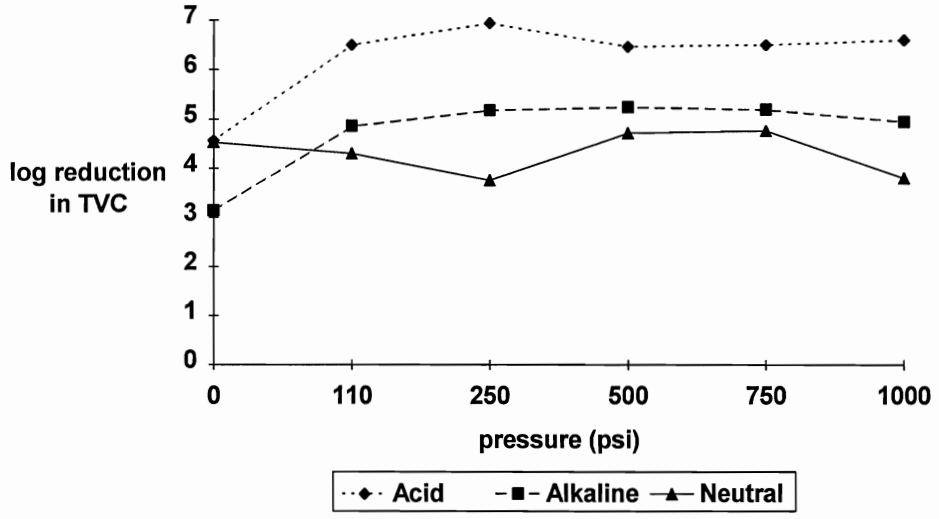


Figure 24 Effect of different detergents and high pressure spraying on the removal of *P.aeruginosa* biofilm

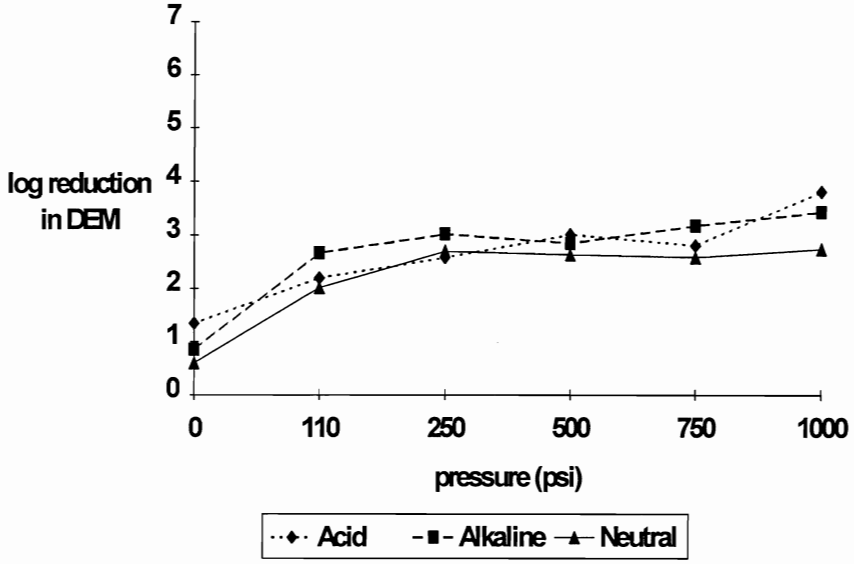
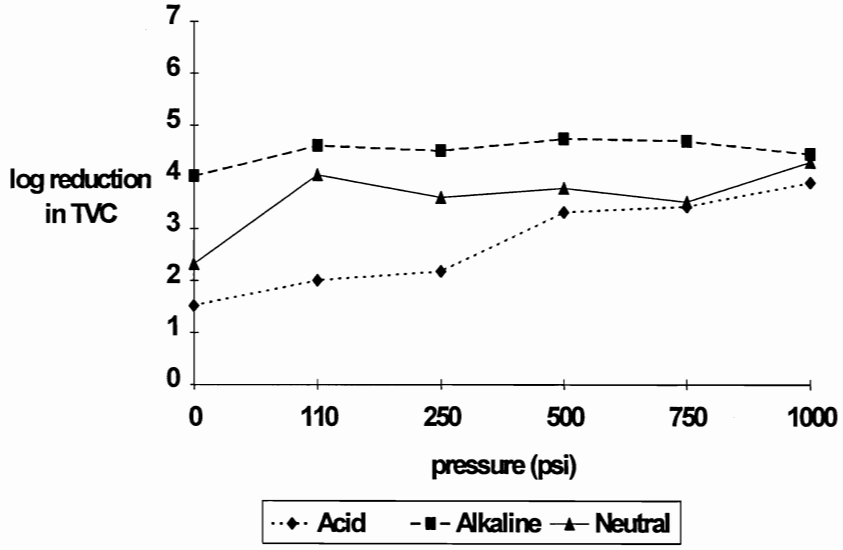


Figure 25 Effect of different detergents and high pressure spraying on the viability of *P.aeruginosa* biofilms



Effectiveness of different cleaning techniques in the removal of factory biofilms

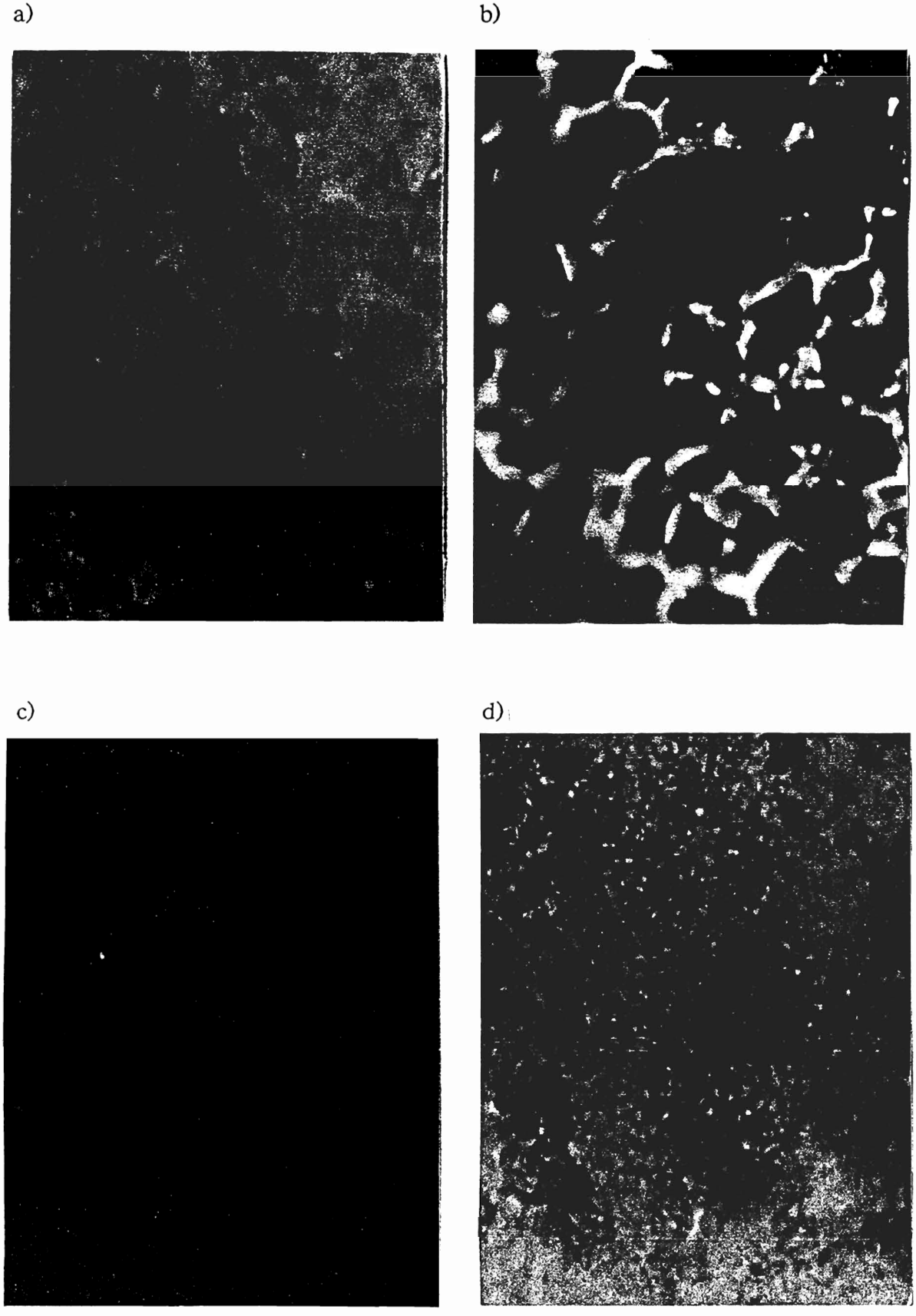
Table 2 compares the efficiency of a range of cleaning methods in terms of the removal of an industrial biofilm. Biofilms were generated in a blancher extractor system found to produce extensive biofilms and were therefore naturally occurring biofilms. The results show that 98% coverage was observed on the control untreated biofilms with a total viable count of 1.73 x 10^8 cfu/cm². Figure 26 shows acridine orange stained surfaces and illustrates the level of organisms remaining on the surfaces after cleaning by differnt methods and highlights the relative removal efficiencies of the methods. The application of a gel followed by a low pressure rinse resulted in a small decrease in coverage (Figure 26a) but had little effect on the viability of the attached population. The use of the detergent and the low pressure rinse may have removed extracellular material surrounding the attached population, thereby reducing the area covered without affecting the viable count. The clean routinely performed by the factory was relatively ineffective as there was little reduction in either total viable count or area covered (Figure 26d). It is difficult to gain access to the extractor system and perhaps this is the reason for the relatively poor clean. The mechanical floor scrubber was very effective, reducing the area coverage to less than 1% (Figure 26b) and the viable count to approximately 10^4 cfu/cm². Sanitation programmes are a combination of four major factors: mechanical/kinetic energy, chemical energy, temperature/thermal energy and time. Mechanical or kinetic energy is employed to physically removal soils and the techniques used include manual brushing, pressure jet washing or the recirculation of fluid in CIP systems. Chemical energy is fundamental to both the cleaning and disinfection elements of the sanitation regime. Cleaning chemicals are used to break down soils so that they can be rinsed away. A combination of these four factors is used in any cleaning system. The input of mechanical/kinetic energy in using the mechanical scrubber was particularly effective at removing the biofilm. Similarly the high pressure spray wash, which also utilises a high level of kinetic energy, reduced the area coverage to less than 1%. Figure 26c shows the level of contamination remaining after the high pressure spray. It is important to consider the possible spread of contamination by these cleaning techniques. Although the high pressure spray resulted in a considerable reduction in the surface population, these systems have been shown to generate aerosols, potentially containing viable microorganisms, over a wide area (Holah *et al* 1993a).

TABLE 2

COMPARISON OF THE EFFECTIVENESS OF A RANGE OF CLEANING SYSTEMS

	Mean Percentage Coverage (± 1 S.D.)	Mean TVC cfu/cm²
Control untreated biofilms	98.4 (3.4)	$1.7x10^8$
Gel detergent plus low pressure rinse	82.7 (18.5)	$1.6x10^8$
Mechanical floor scrubber	0.4 (0.4)	$8.9x10^4$
High pressure spray wash	0.1 (0.0)	$6.6x10^5$
Factory clean (low pressure wash, disinfection, rinse)	95.1 (3.6)	$1.5x10^8$

Figure 26 Acridine orange stained surfaces showing the levels of micro-organisms remaining after cleaning with a) gel plus low pressure rinse, b) mechanical floor scrubber, c) high pressure spray and d) the factory clean.



To be replaced with colour photographs

Effect of the presence of soil on the removal of biofilms

In the factory environment, bacteria and product residues will be present on the surfaces. The following results show the effect of the presence of soil on the removal of bacterial biofilms.

Figure 27 shows the effect of the presence of bovine albumin or yeast extract on the removal of *P.aeruginosa* biofilms. These results show that the presence of soil did not affect the removal of *P.aeruginosa* as the extent of removal is not significantly different from that observed in the absence of soil (Figure 3).

The DEM results show that the degree of removal of *P.aeruginosa* was similar whether the biofilm was freshly developed or dried on, or whether soil was present or absent.

The log reductions (as measured by TVC) of dried-on *P.aeruginosa* biofilms in the absence of soil were approximately one log order (Figure 16); however, the log reductions of dried-on biofilms observed in the presence of soil were higher at approximately four to five log orders (Figure 28). In contrast, similar log reductions (as measured by TVC) were observed for freshly developed biofilms in the presence and absence of soil (Figures 14 and 28). This would seem to suggest that the presence of soil dramatically affected the viability of the dried-on *P.aeruginosa* biofilm, although the mechanism is unclear.

Figures 29 and 30 shows the effect of the presence of soil on the removal of *S.aureus* biofilms as measured by DEM and TVC respectively. The log reductions as measured by DEM of the freshly developed biofilms in the presence of soil are not significantly different (5% significance level) from those shown in Figure 16 (absence of soil). In contrast, the removal of dried-on *S.aureus* biofilms is reduced by the presence of soil by one log order. In the absence of soil the reductions achieved by spraying were approximately 2-2.5 log orders; however, in the presence of soil, and in particular yeast extract, the reductions were approximately 1-1.5 log orders. This would seem to suggest that the presence of soiling material makes the removal of dried-on *S.aureus* more difficult, possibly by protecting the attached cells.

Figure 27 Effect of the presence of soil (bovine albumin, ba or yeast extract, ye) on the removal (DEM) of freshly developed or dried on (do) *P.aeruginosa* biofilms

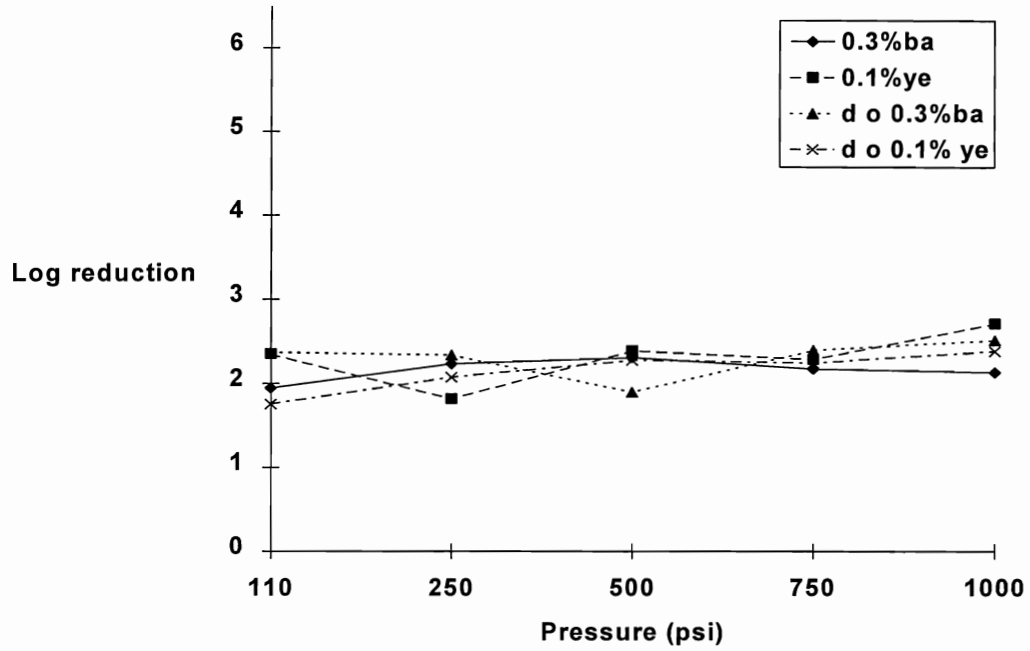


Figure 28 Effect of the presence of soil (bovine albumin, ba or yeast extract, ye) on the removal (TVC) of freshly developed or dried on (do) *P.aeruginosa* biofilms

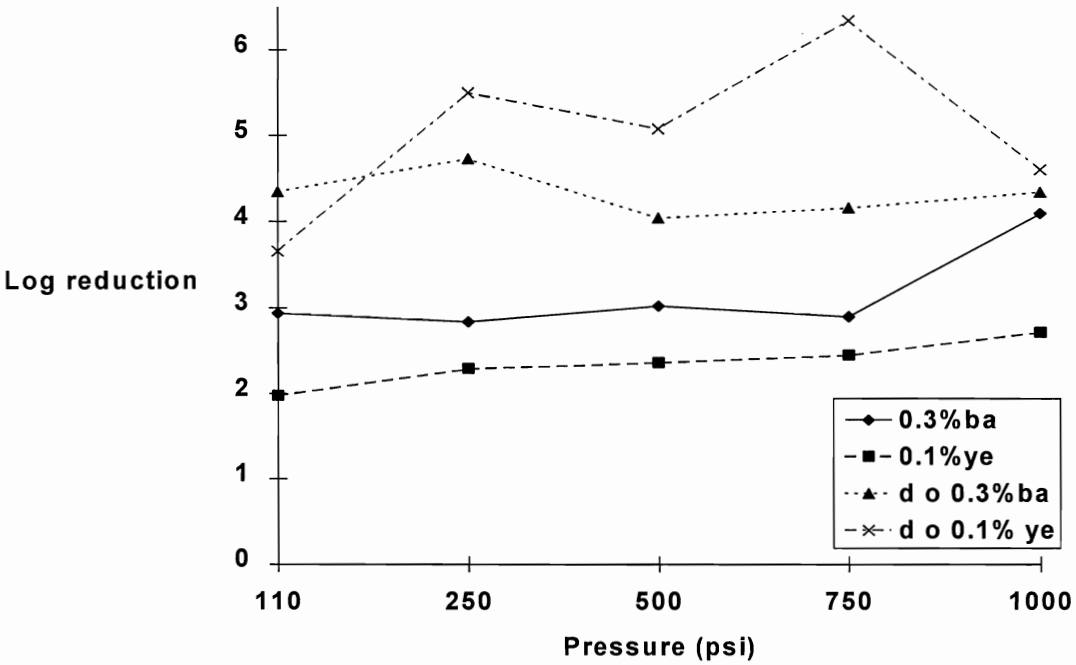


Figure 29 Effect of the presence of soil (bovine albumin, ba or yeast extract, ye) on the removal (DEM) of freshly developed or dried on (do) S.aureus biofilms

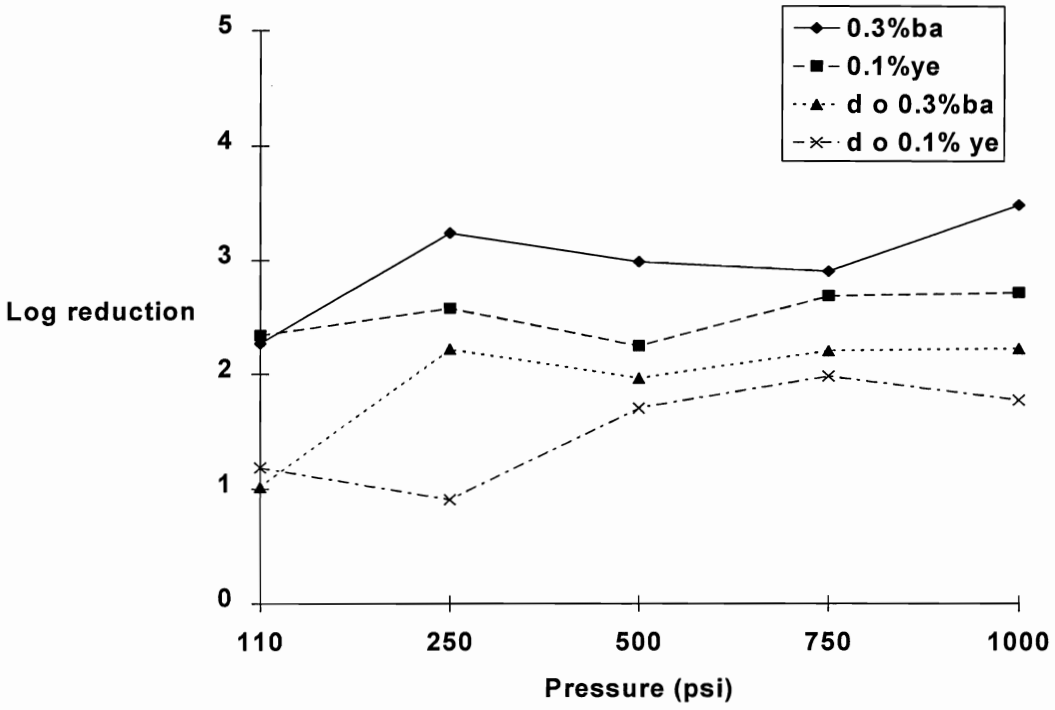
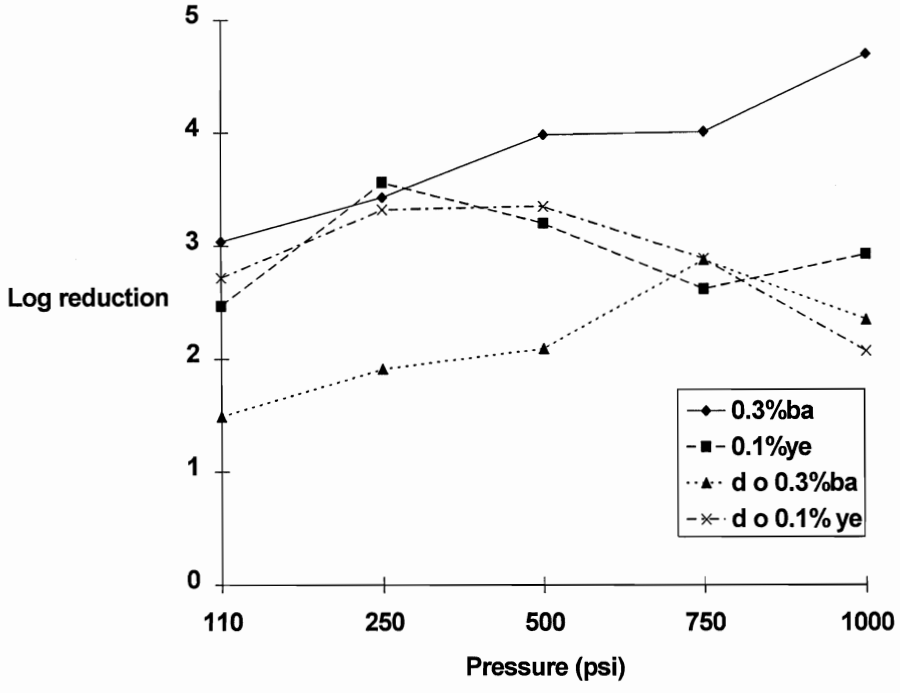


Figure 30 Effect of the presence of soil (bovine albumin, ba or yeast extract, ye) on the removal (TVC) of freshly developed or dried on (do) *S.aureus* biofilms



Influence of CIP flow velocity and cleaning time on the removal and viability of biofilms

The flow velocity and cleaning time are of critical importance in a clean-in-place programme. Figure 31 shows the effect of flow velocity on the removal of *P.aeruginosa* biofilms. The DEM counts show that increasing flow velocity from 0.25m/s to 1.0m/s did not result in an increase in the removal of the biofilm. However, increasing the flow rate above 1m/s (up to 3m/s) did result in a significant increase in the removal of attached *P.aeruginosa* (significantly different at 2.25 and 3.0 m/s) to a log reduction of approximately two. The TVC data reflects both removal of attached cells, and loss in viability of the attached cells due to the high temperatures and the presence of the detergent. In contrast to the DEM results, increasing the flow rate did not have a significant effect on the log reductions as measured by TVC, although the log reductions were higher due to a loss in viability.

Figure 32 shows the effect of cleaning time on the removal of *P.aeruginosa* biofilm. Analysis of the DEM data shows that there was no significant increase in the removal of *P.aeruginosa* biofilms with longer cleaning times. Differences were observed in the viability of the attached population. Analysis of the TVC data showed that increasing the cleaning time from 10 to 20 minutes resulted in a significant increase in the log reduction (due to the effects of the temperature and detergent on the viability of the attached population), although cleaning times of greater than 20 minutes did not result in any further increase.

The maximum log reduction achieved in these studies was approximately two, and therefore with an initial level of 10^7 cells/cm², 10^5 cells/cm² remained after the cleaning procedure although the viability of this population was reduced by the cleaning temperature and chemical. Timperley (1981) also found that approximately two log orders remained on the surfaces after cleaning at similar velocities as measured by swabbing and TVC analysis. In conclusion, at the temperature tested, bacteria attached to surfaces may remain in a viable form even after the CIP clean. It is recommended that flow velocities in excess of 1.5m/s are used to optimise bacterial removal.

Figure 31 Effect of Clean-in-place flow velocity on the removal of *P.aeruginosa* biofilm using mildly alkaline detergent at 65 °C for 20 minutes

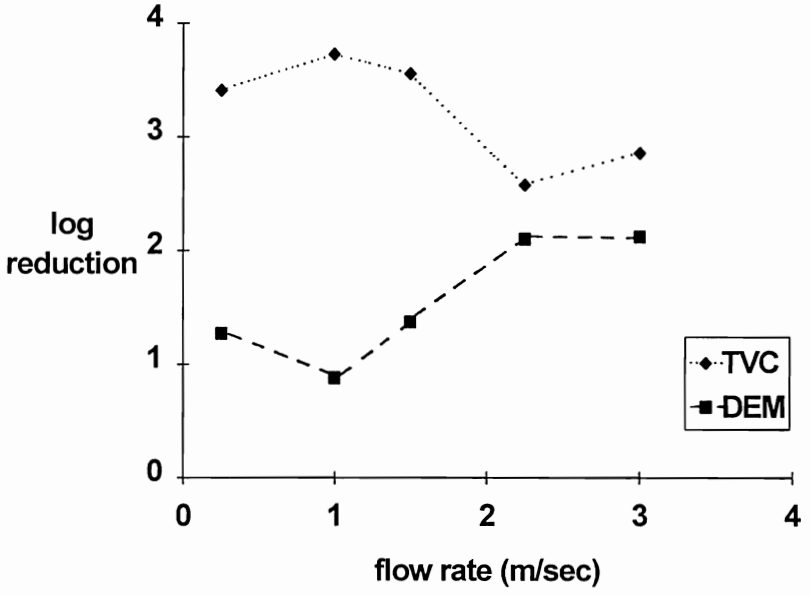
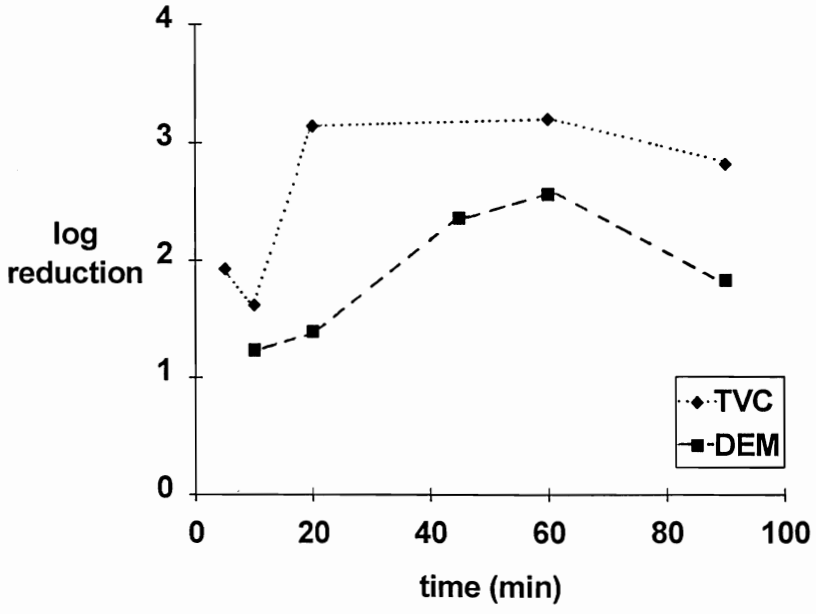


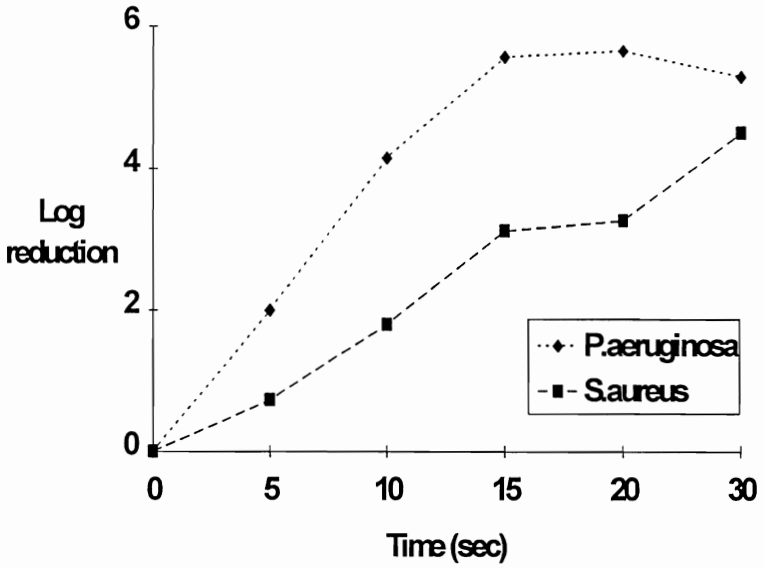
Figure 32 Effect of Clean-in-place cleaning time on the removal of *P.aeruginosa* biofilm using mildly alkaline detergent at 65 °C at 1.5 m/s



Effectiveness of steam cleaning in the disinfection of biofilms

Figure 33 shows the effect of steam time on the viability of attached *P.aeruginosa* and *S.aureus*. The graph shows that the viability of *P.aeruginosa* reduces linearly up to 15 seconds; steaming for times longer than 15 seconds did not increase the log reductions. *S.aureus* biofilms were more resistant to the steam, and the log reductions only approached similar levels to those observed for *P.aeruginosa* after 30 seconds. This is a useful technique for microbial reduction in particular areas such as hard-to-clean crevices, but the application times are too long for general open surface cleaning.

Figure 33 Effect of steaming on the viability of *S.aureus* and *P.aeruginosa* biofilms

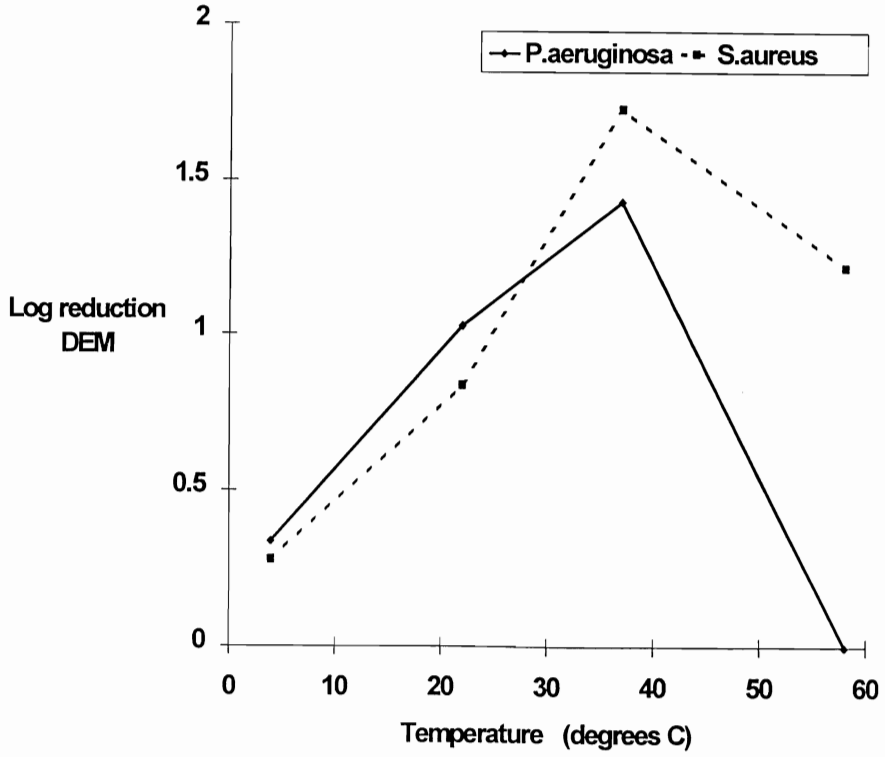


Assessment of factors influencing the disinfection of attached microbes

Effect of temperature on the disinfection of biofilms

Figure 34 shows that increasing the temperature to 37°C increased the log reductions observed. The log reduction appears to be lower at 58°C because the temperature alone reduced the viability of the attached population, so that no added effect was observed with the disinfectant treatment. The graph therefore shows that increasing temperature to 37°C increased the efficiency of disinfection of *S.aureus* and *P.aeruginosa* biofilms with a quaternary ammonium compound. Jeffrey and Matthews (1981) found a similar relationship for *S.aureus* and *P.aeruginosa* in suspension using a household pine disinfectant. The activity of a disinfectant is usually increased when the temperature at which it acts is increased, and Russell (1982) describes this relationship in more detail. The results presented here show that a similar relationship was observed for attached cells. In addition to increasing the activity of the disinfectant, increasing temperature may also increase the detachment of cells from the surface as found by Lewis *et al* (1989). The importance of this relationship relates to the temperature of the food processing environment. Chilled food factories, for example, operate at temperatures below ambient and therefore the efficiency of the disinfectant may be reduced.

Figure 34 Effect of temperature on the disinfection of *P.aeruginosa* and *S.aureus* biofilms



Effect of detergent treatment on the disinfection of biofilms

Various studies have shown that microbes show increased resistance to disinfectants when attached to surfaces. Studies using impedimetric and conductance based techniques have demonstrated the increased resistance of attached microbes. Holah *et al* (1990a) found that *P.aeruginosa, S.aureus* and *Proteus mirabilis* were 10-100 times more resistant to a range of biocides when surface attached. Dhaliwal *et al* (1992), using impedimetric techniques, showed that *S.aureus, Escherichia coli, Salmonella enteritidis* and *Listeria monocytogenes* were more resistant to disinfectants when attached to a variety of surfaces.

Disinfectants are commonly assessed using suspension-based tests; however, bacteria attached to surfaces are more resistant to disinfectants. Bacteria attached to surfaces in the food processing environment will be exposed to a variety of environmental stresses, such as elevated temperatures, detergents, desiccation and nutrient limitations before treatment with disinfectant products. For this reason concentrations of disinfectant that are not active against 'healthy' rapidly growing cells, may be effective against physiologically stressed cells that have been exposed to the above mentioned environmental stresses.

Figure 35 shows the effect of detergent type on the disinfection of *P.aeruginosa* biofilms. Figure 35 (a) shows that the neutral detergent alone had some biocidal effect and results in log reductions similar to those observed for the biguanide product. The QAC produces a 5 log reduction, but the treatment of the *P.aeruginosa* biofilm with the neutral product prior to disinfection decreases the effectiveness. Figure 35 (b) shows that the alkaline detergent is a very effective biocide on its own, but detergent treatment followed by disinfection is particularly effective with both disinfectant products, even the biguanide which is relatively ineffective on its own. The acidic product inhibits the effect of the QAC, but enhances the action of the biguanide (Figure 35 (c)).

Figure 36 shows the effect of the three detergents on the disinfection of *S.aureus* biofilms. Generally similar trends were observed as those described for *P.aeruginosa.*

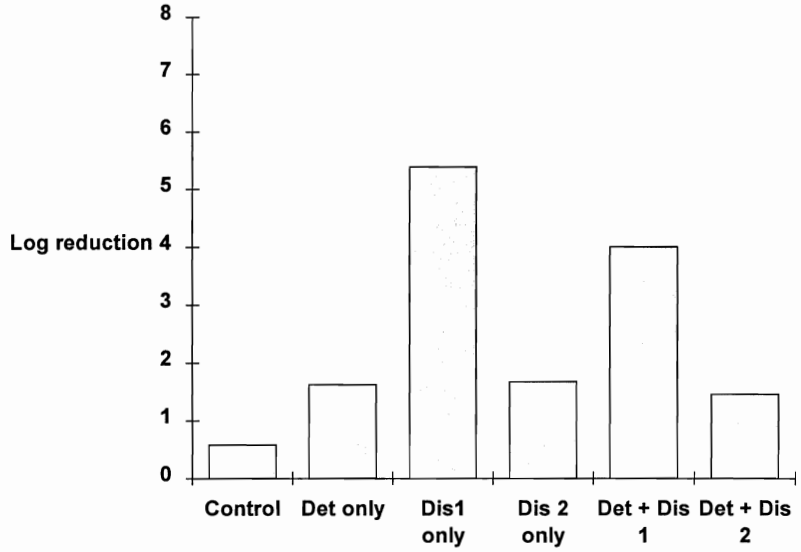
Figure 37 shows the effect of detergent treatment on the disinfection of multilayer biofilms. These multilayer biofilms comprise a matrix of cells and extracellular polymer material. This exopolymer material is thought to protect the cells from disinfection.

Comparison of Figure 37 and Figure 35 shows that very similar results were obtained for multilayer and monolayer *P.aeruginosa* biofilms; however, as the concentration of cells per cm^2 was higher in the multilayer biofilms, a higher number of cells remained after disinfection.

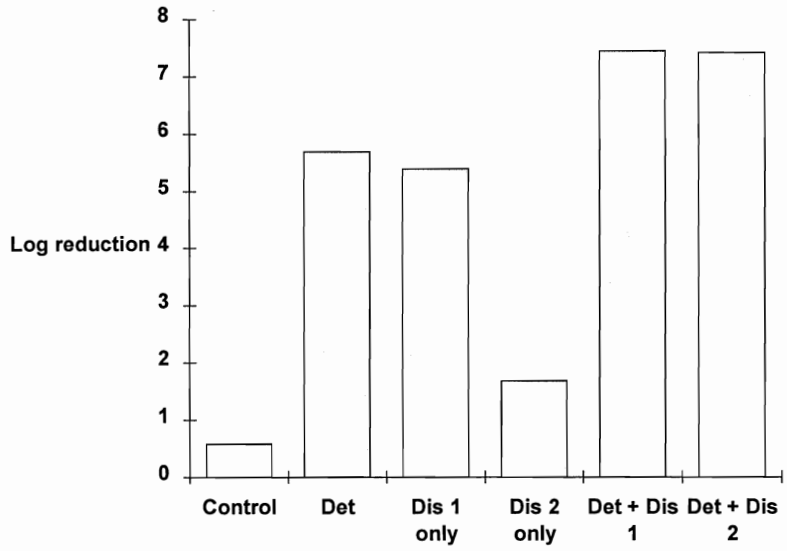
Figure 38 shows the effect of detergent treatment on the disinfection of *S.aureus* multilayer biofilms, and the detergent treatment and disinfection patterns were similar to those observed for monolayer biofilms.

Figure 35 Effect of detergent treatment on the disinfection of *P.aeruginosa* biofilm using either a quaternary ammonium compound (Dis 1) or a biguanide (Dis 2)

(a) Neutral



(b) Alkaline



(c) Acidic

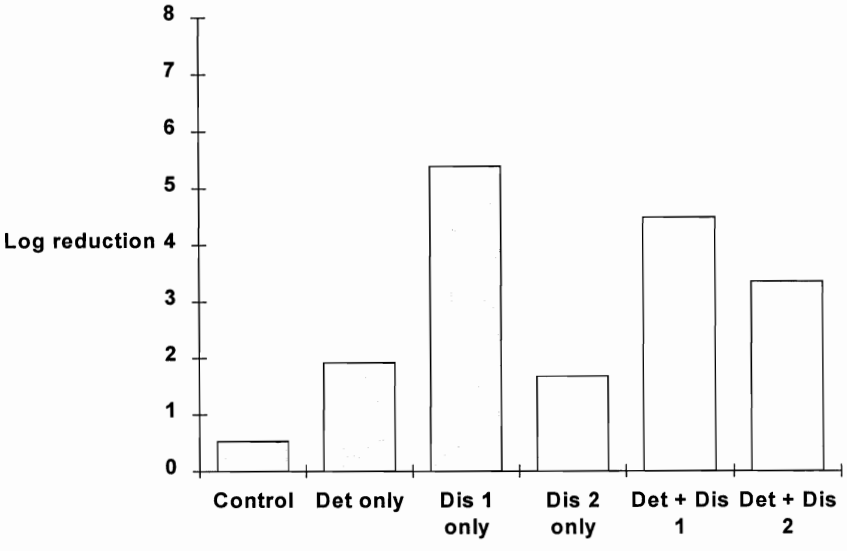
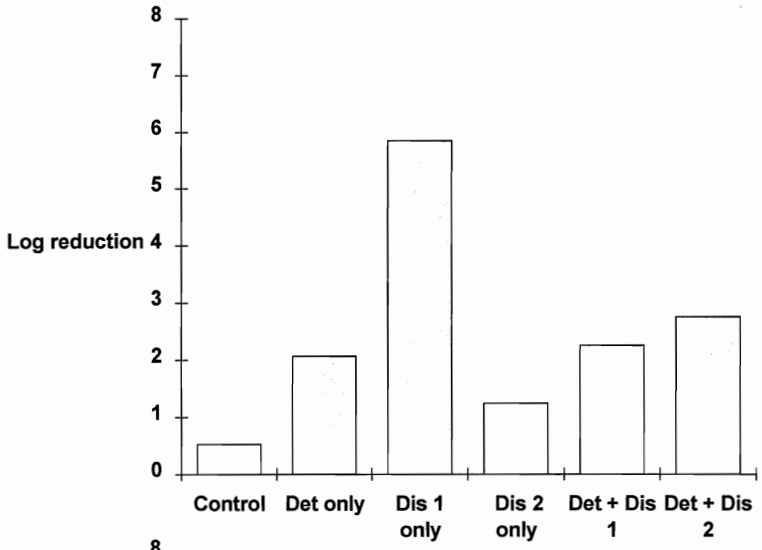
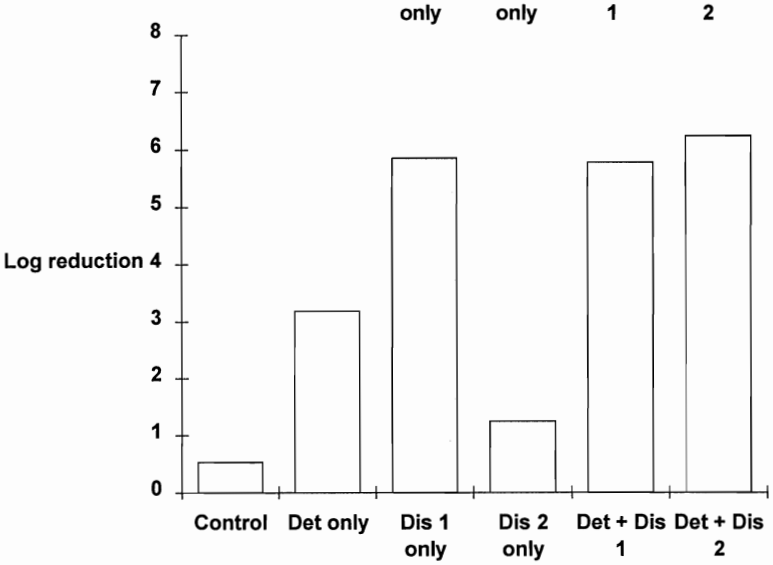


Figure 36 Effect of detergent treatment on the disinfection of *S.aureus* biofilm using either a quaternary ammonium compound (Dis 1) or a biguanide (Dis 2)

(a) Neutral



(b) Alkaline



(c) Acid

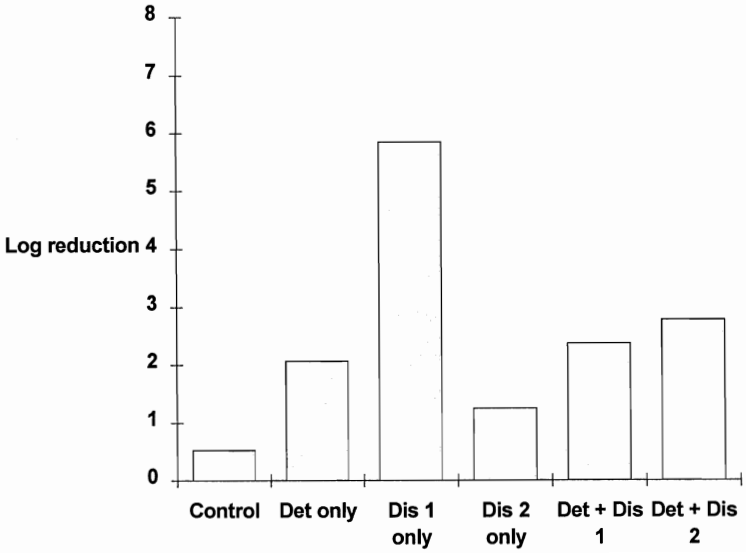
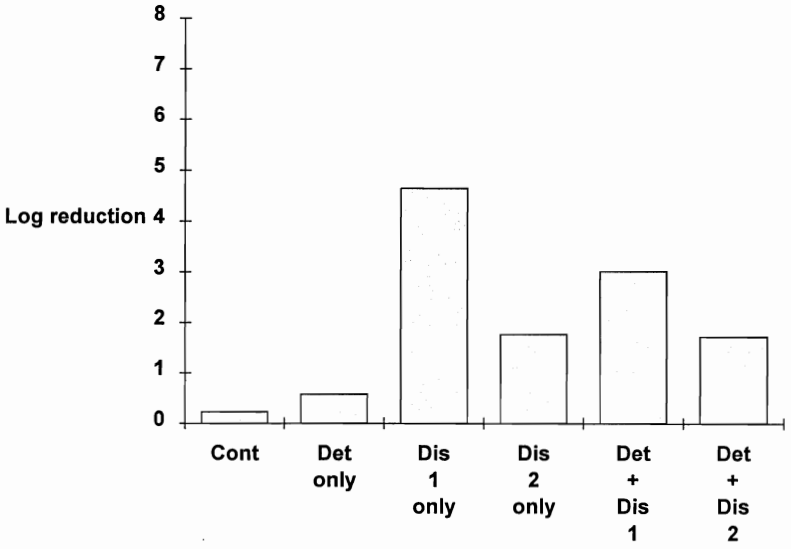
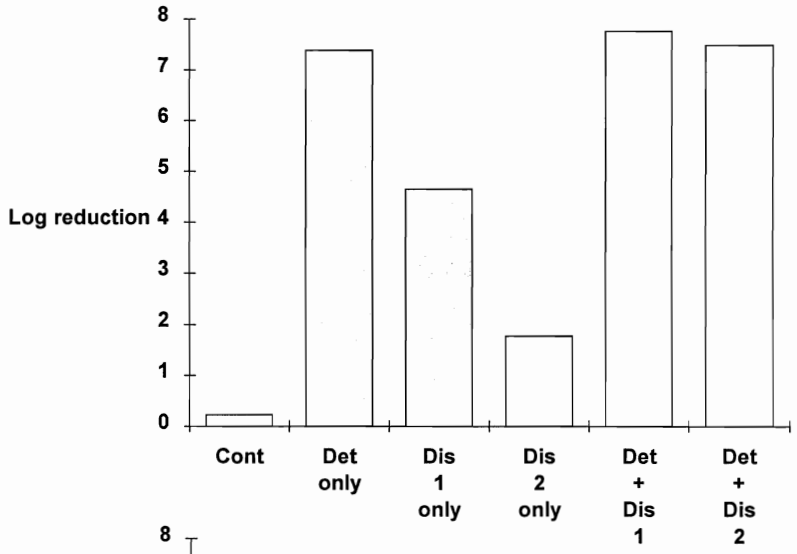


Figure 37 Effect of detergent treatment on the disinfection of *P.aeruginosa* multilayer biofilms using a quaternary ammonium compound (Dis 1) and a biguanide (Dis 2)

(a) Neutral



(b) Alkaline



(c) Acid

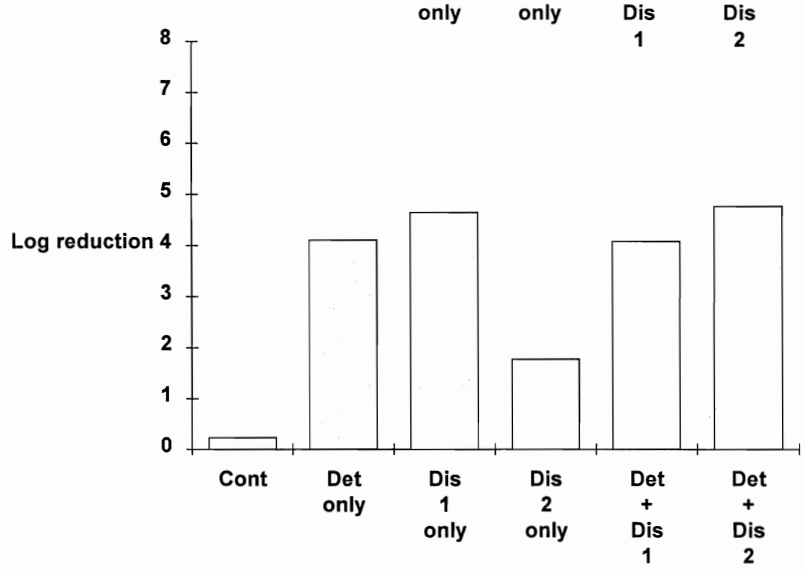
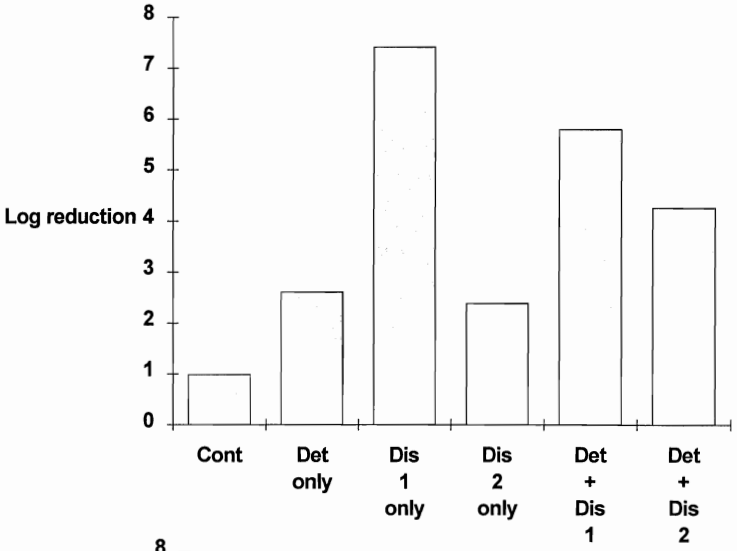
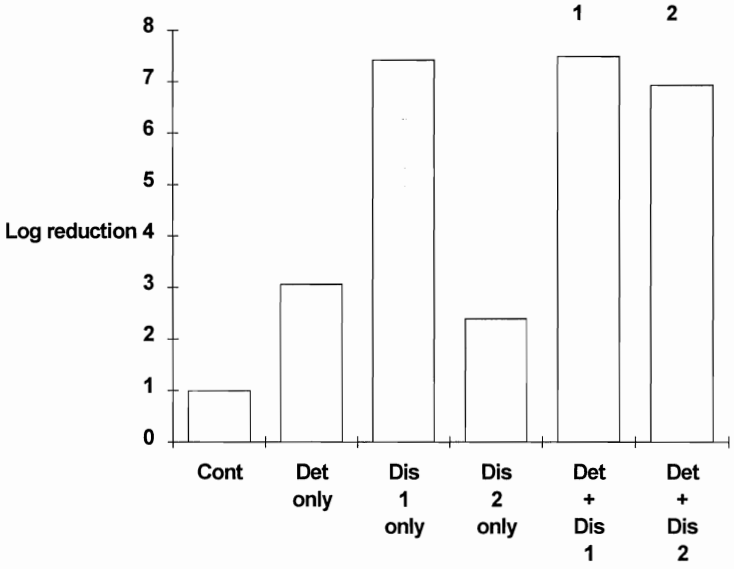


Figure 38 Effect of detergent treatment on the disinfection of *S.aureus* multilayer biofilms using a quaternary ammonium compound (Dis 1) and a biguanide (Dis 2)

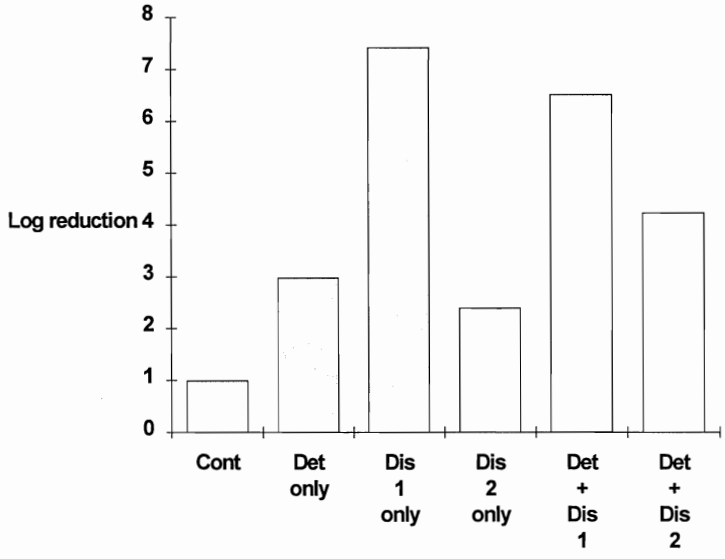
(a) Neutral



(b) Alkaline



(c) Acidic



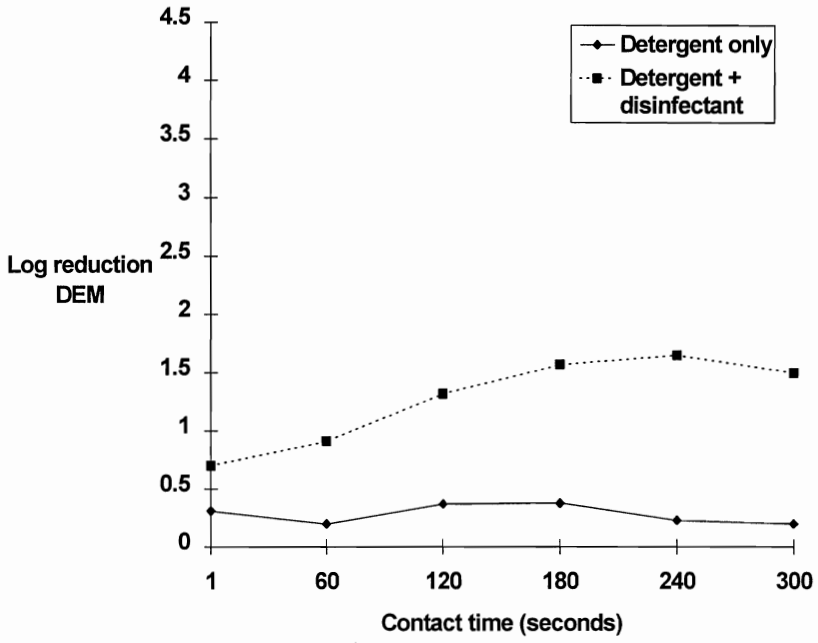
The results in Figures 39 and 40 show the effect of detergents on the disinfection of *P.aeruginosa* and *S.aureus* biofilms as measured by a conductance technique. The advantage of this technique is that it allows enumeration of attached microorganisms *in situ* without removing them from the surface. If cells are stressed or injured then they will grow more slowly and so longer detection times will be observed, and therefore the Malthus system gives information about stressed and damaged cells. The disadvantage is that longer detection times can indicate low numbers or higher numbers of stressed or injured cells. In the case of *P.aeruginosa* the disinfectant produced only a one log reduction in the attached cells. Figures 39 (a) and (c) show that the neutral and alkaline detergents did not achieve log reductions greater than one. In contrast the acidic detergent (Figure 39 (b) and particularly the strongly alkaline (Figure 39 (d)) produced log reductions of one and four respectively and were therefore biocidal. In general disinfectant treatment after detergent treatment resulted in a further one log reduction above that observed for detergent alone. These results show that an additive effect was observed, so that the biocidal effect due to certain detergents increases the net log reductions achieved. Various workers have shown that bacteria attached to surfaces are more resistant to biocides (Holah *et al*, 1990a; Dhaliwal *et al*, 1992; Le Chevalier *et al*, 1988); however, in the food processing environment bacteria attached to surfaces are exposed to a variety of physiological stresses such as detergent treatment that influence the susceptibility to disinfectants.

Figure 40 shows the results obtained for *S.aureus* The disinfectant alone produced a 1.25 log reduction in attached *S.aureus.* The strongly alkaline detergent (Figure 40 (d)) produced log reductions in excess of 2.5 after 60 seconds; however, the other detergents had little bactericidal action. Treatment of *S.aureus* biofilm with the acidic and strongly alkaline detergents (Figure 40 (b) and (d) respectively), followed by disinfectant treatment, enhanced the log reductions to more than 2 and 3.5 respectively. However, the neutral and alkaline products did not enhance the disinfection.

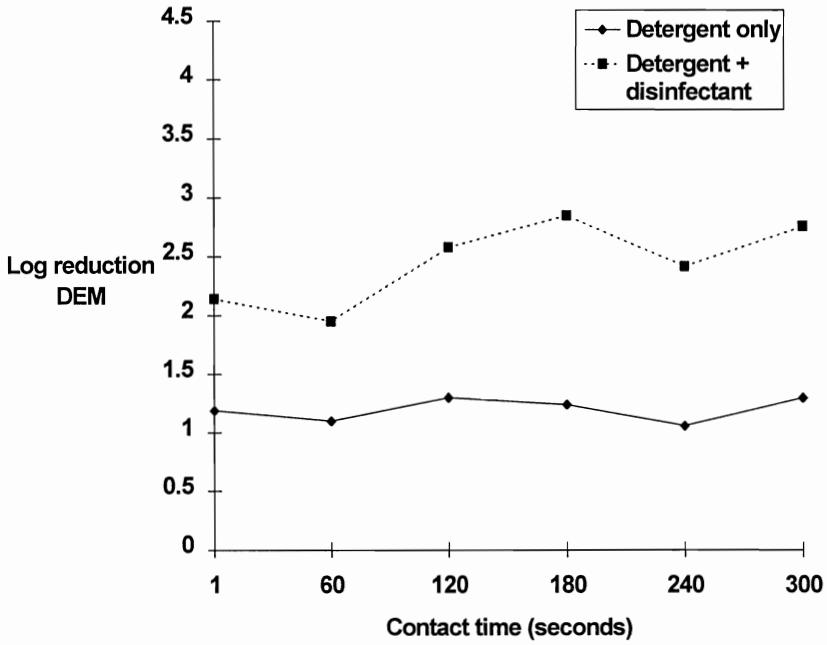
Biofilms are complex structures comprising both microbes and their exopolymers and therefore biocides may be ineffective due to problems of disinfectant reaching the microbes. Figures 35 - 40 show that detergent action may have an additive biocidal effect, and therefore disinfectants that do not produce high log reductions alone may produce acceptable log reductions in conjunction with certain detergents. There is also some evidence that longer contact times result in greater log reductions.

Figure 39 Effect of detergents on the disinfection of *Paeruginosa* biofilm as measured by conductance (the disinfection alone produced a 1.0 log reduction after a contacrt time of 5 minutes)

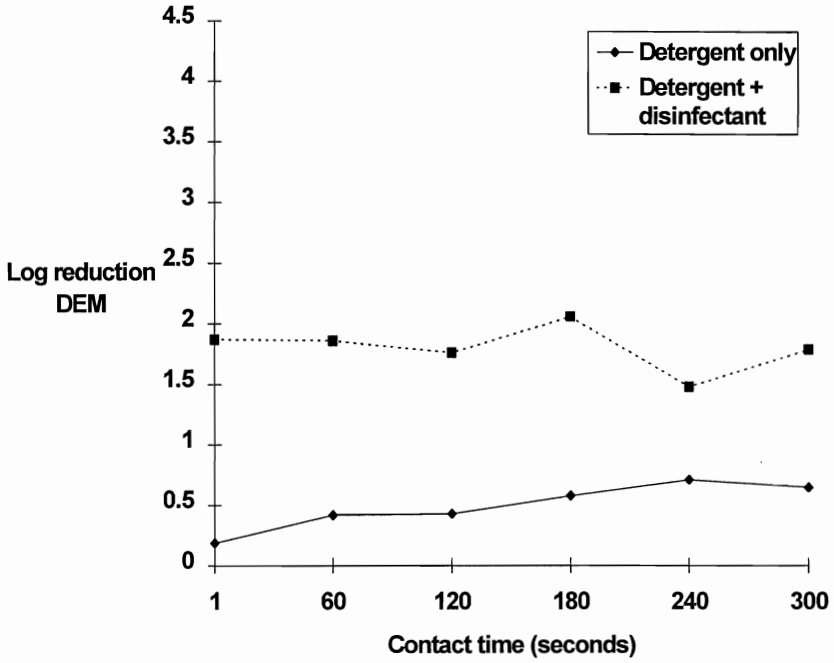
(a) Neutral (pH 8.3)



(b) Acidic (pH 1.7)



(c) Alkaline
(pH 11.6)



(d) Strongly Alkaline
(pH 12.7)

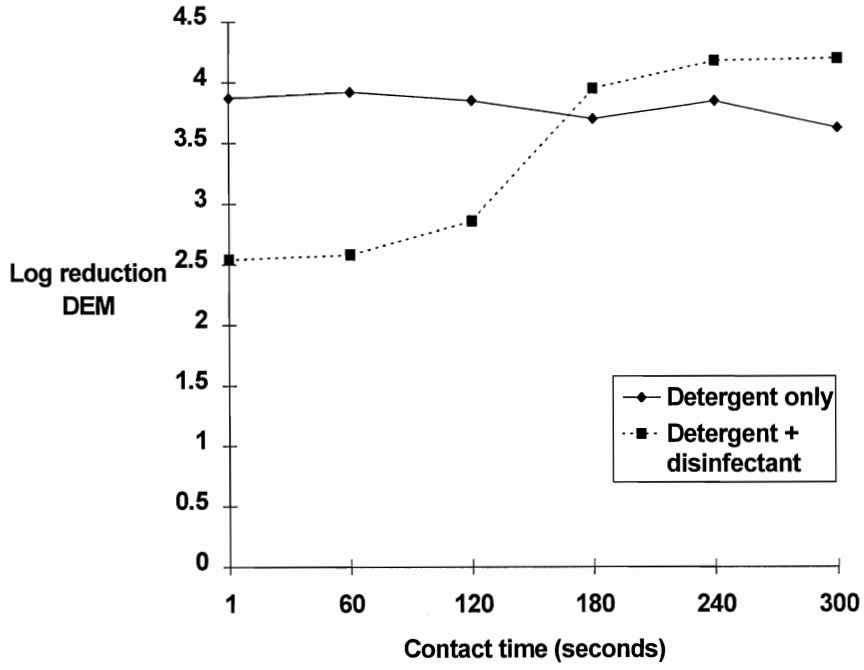
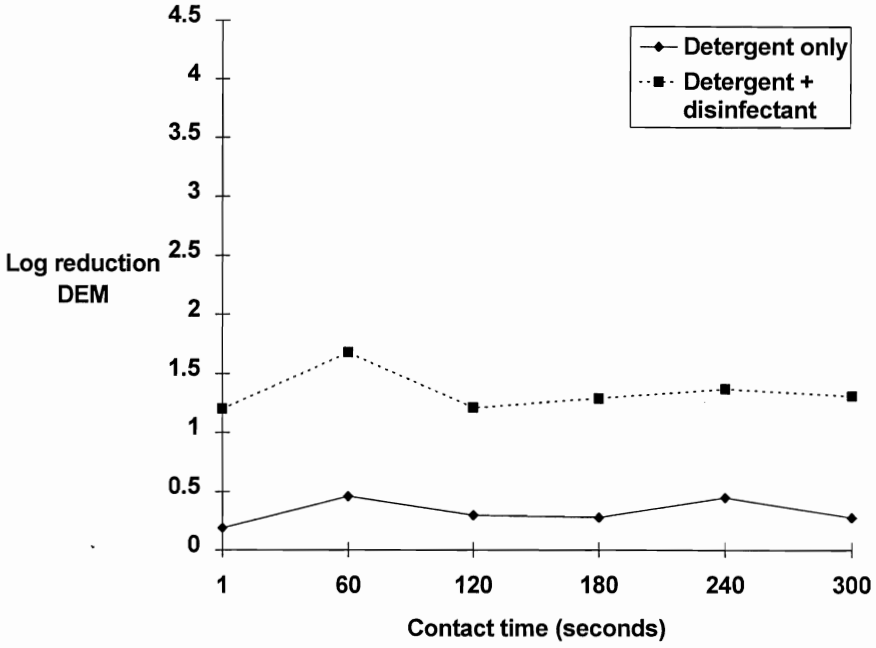
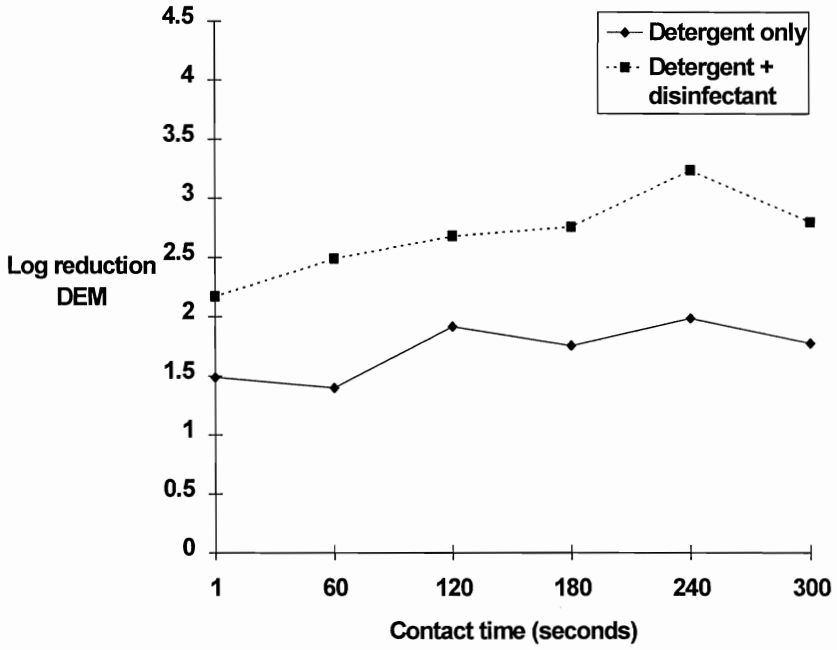


Figure 40 Effect of detergents on the disinfection of *S.aureus* biofilm as measured by conductance (the disinfection alone produced a 1.25 log reduction after a contact time of 5 minutes)

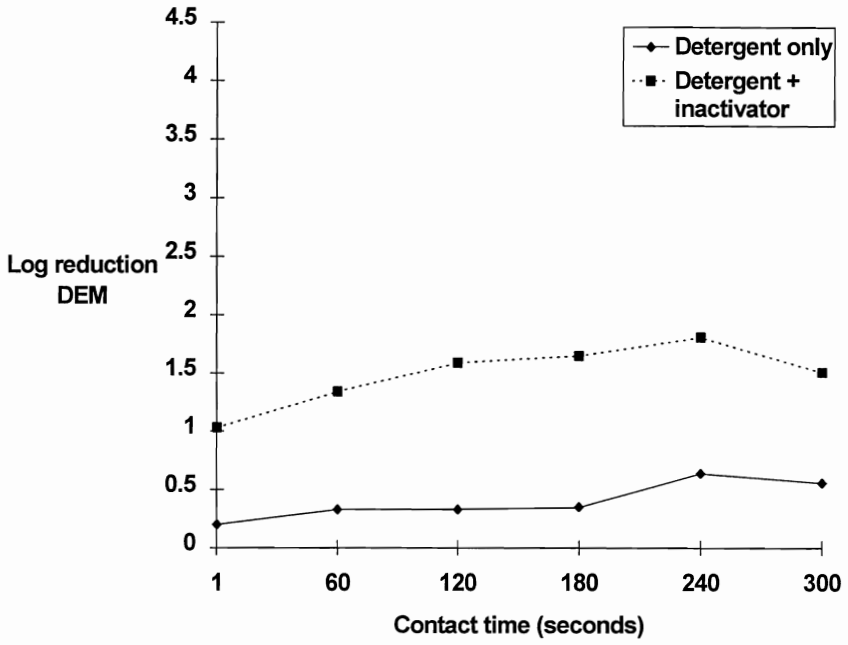
(a) Neutral (pH 8.3)



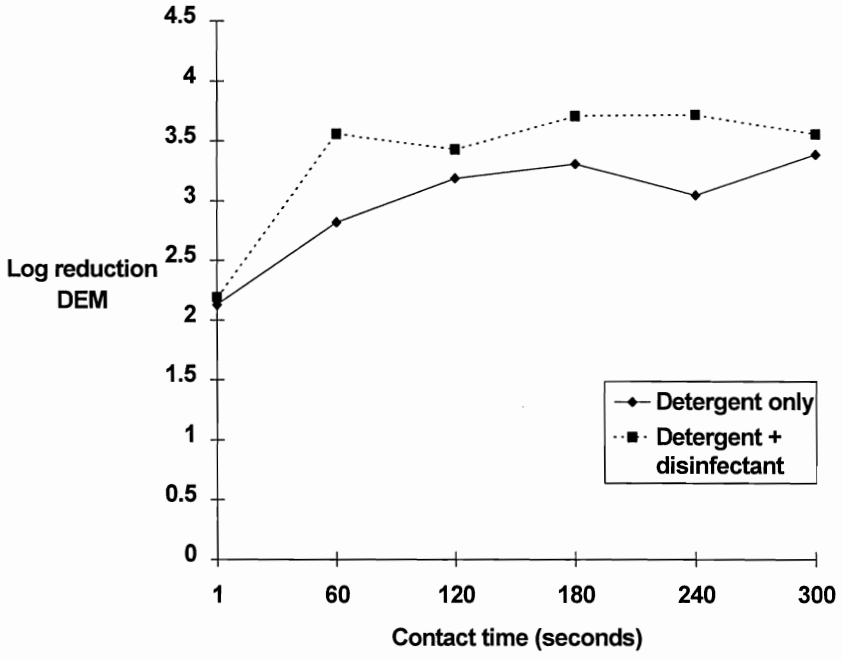
(b) Acidic (pH 1.7)



(c) Alkaline (pH 11.6)



(d) Strongly Alkaline (pH 12.7)



Evaluation of the effect of environmental conditions on biofilms

Effect of pretreatment of the surfaces on the attachment of bacteria

Figure 41 shows the effect of the presence of various soils on the attachment of *P.aeruginosa.* Attachment to the surfaces covered with dried-on milk and albumin was significantly lower than control clean stainless steel or other treatments. Presence of soil therefore inhibited attachment in the case of dried-on milk or albumin whilst the other treatments did not promote the attachment of *P.aeruginosa.*

Figure 42 shows the effect of the presence of various soils on the attachment of *S.aureus.* In the case of this organism treatment of the stainless steel surfaces with milk or dried-on milk significantly reduced the attachment of *S.aureus.*

Similarly, Helke *et al* (1993) found that skim or whole milk inhibited the attachment of *Listeria monocytogenes* and *Salmonella typhimurium* and attributed the inhibition of attachment to the major proteins.

Figure 41 Effect of pretreatment of the surface with milk, dried on milk, bovine albumin (ba), dried on BA and yeast extract and dried on yeast extract on the attachment of *P.aeruginosa*

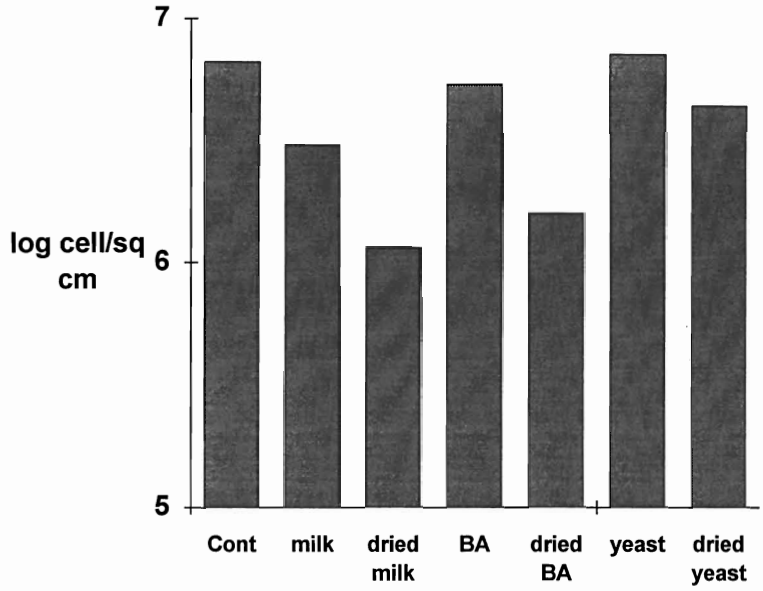
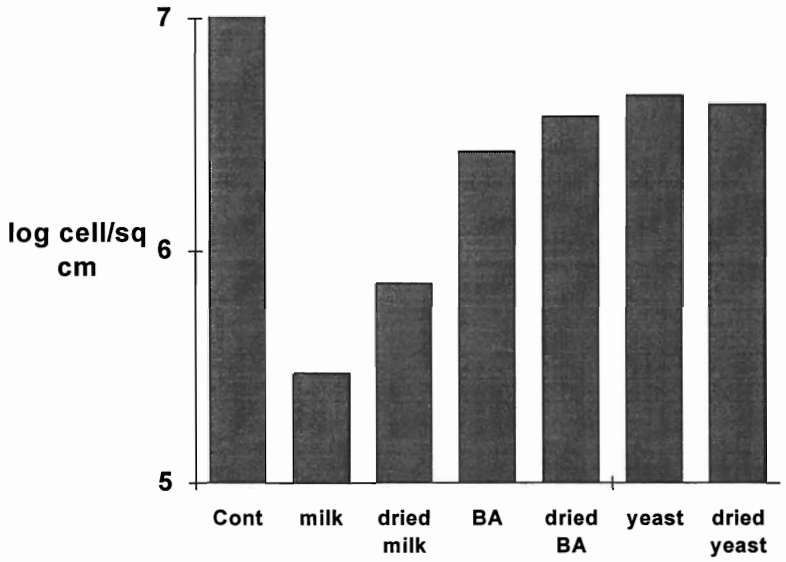


Figure 42 Effect of pretreatment of the surface with milk, dried on milk, bovine albumin (BA), dried on BA, yeast extract or dried on yeast extract on the attachment of *S.aureus*



Effect of humidity on biofilms

Figure 43 shows the effect of humidity on the survival of *P.aeruginosa* cells in a biofilm. The graph shows that there was no significant change in the surface population of the coupons kept in 100% humidity for the 9-day period. However, lower humidity levels (14, 34 and 76%) resulted in a loss in viability of the attached population and within 3 days the viability of the attached population had been reduced by 3 log orders.

S.aureus biofilms showed very different results (Figure 44). This organism was particularly resistant to desiccation and even after 9 days there was in excess of 10^5 cells/cm^2 present on the stainless steel coupons. *S.aureus* is tolerant of low water activities and high salt concentrations and therefore desiccation resistance is not unexpected.

The relevance for the factory environment is that if biofilms are allowed to develop and are not removed then even in the absence of visible moisture the bacteria may survive for long periods and remain as a source of contamination.

Figure 43 Effect of humidity on the survival of *P.aeruginosa* in biofilms

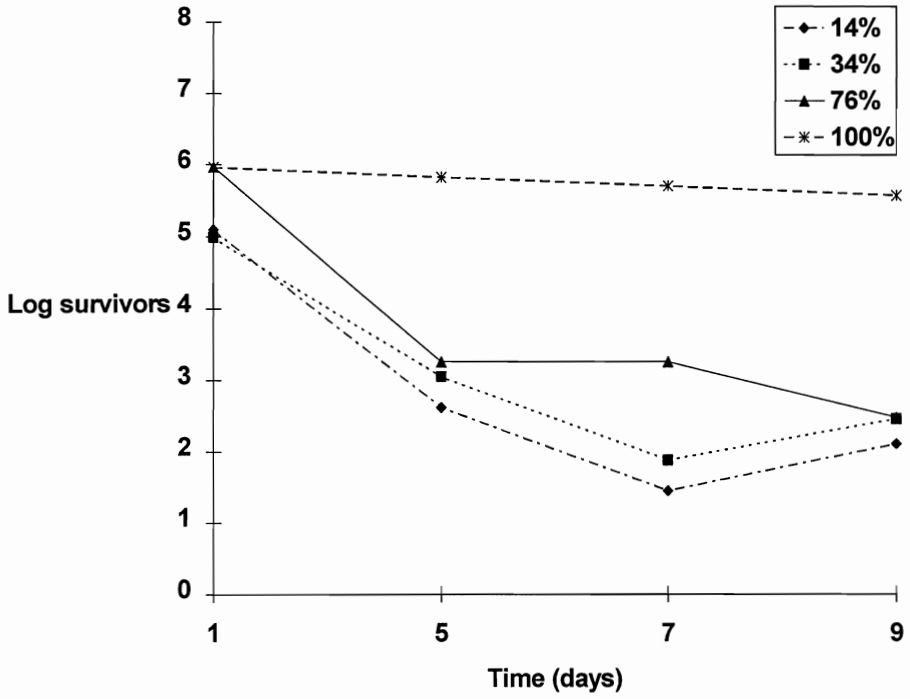
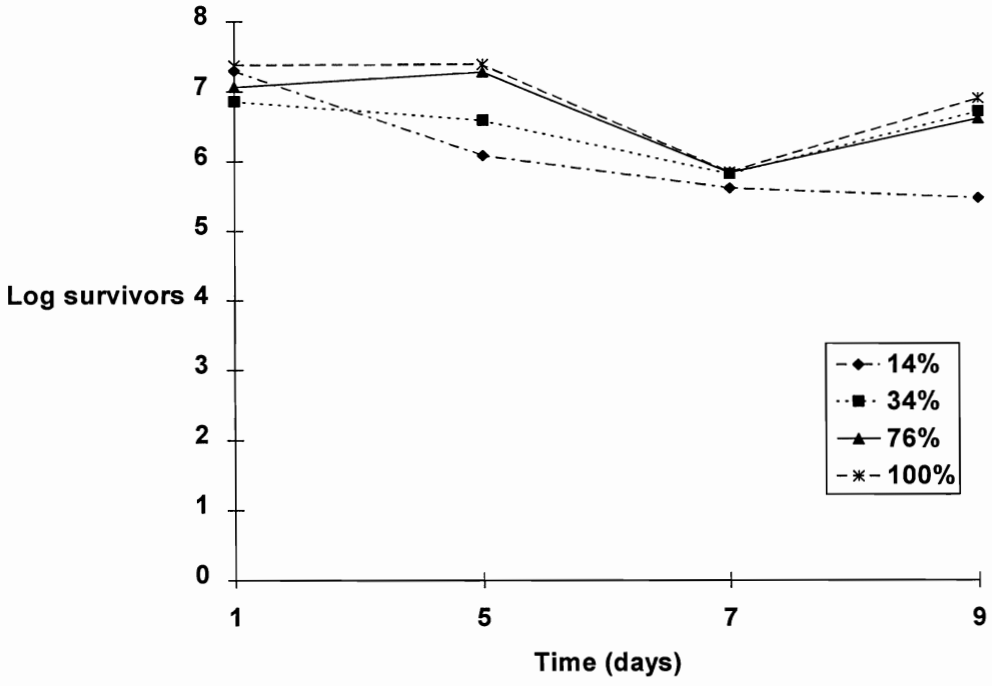


Figure 44 Effect of humidity on the survival of *S.aureus* in biofilms

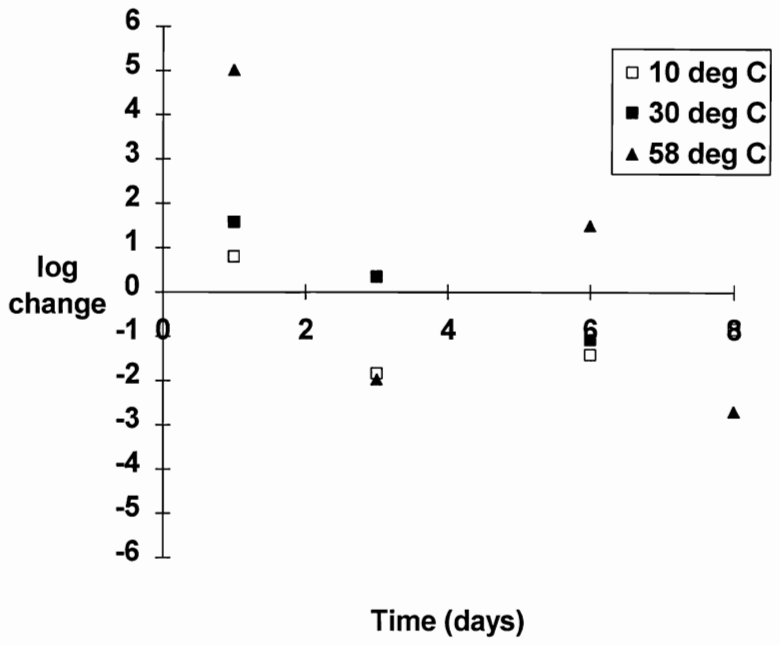


Effect of temperature and humidity on factory biofilms

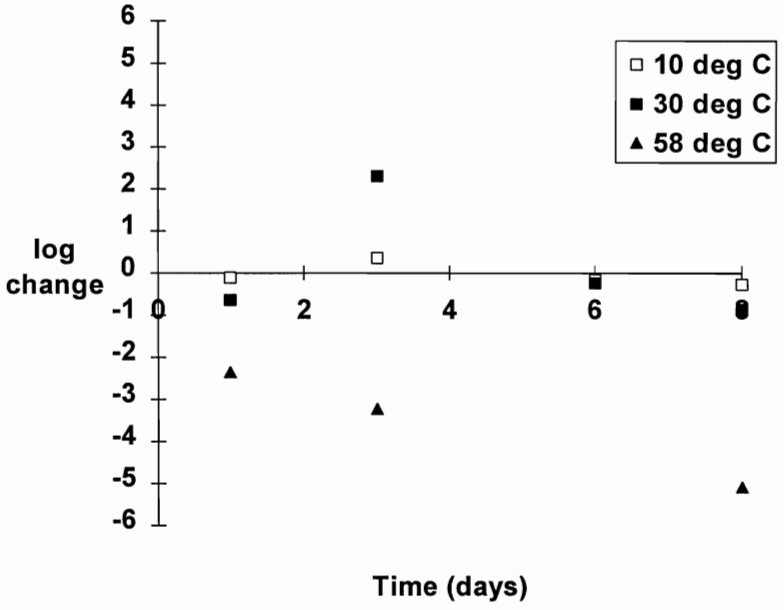
The effects of temperature and humidity on biofilms developed in the blancher extractor system were examined by placing stainless steel coupons with attached biofilm into chambers with different humidities at different temperatures. The initial total viable count was approximately 10^6 cfu/cm² with a thermophile count of approximately 10^2 cfu/cm². The results obtained are shown in Figure 45. Figure 45 (a) shows that after one day at 14% relative humidity, growth was observed at all temperatures, particularly at 58°C due to the presence of thermophiles. However, at longer exposure times to the low humidity no change in the viable population (or a log reduction) was observed. At 30% and 75% relative humidity there was generally a reduction in the viability of the attached populations. At 100% relative humidity growth was observed at all temperatures, although at 8 days limited growth was observed. The temperature that produced highest levels of growth was 30°C. The temperature of the blancher extractor system was 27°C and therefore this temperature and humidity combination (30°C and 100% respectively) was closest to the natural environment for these biofilms. These results suggest that at high humidities bacteria may grow on surfaces and can survive for at least 8 days.

Figure 45 Effect of temperature (10, 30 or 58°C) and relative humidity (14, 33, 75 and 100%) on the viability of factory biofilms (Positive log change indicates growth and negative log change indicates loss in viability)

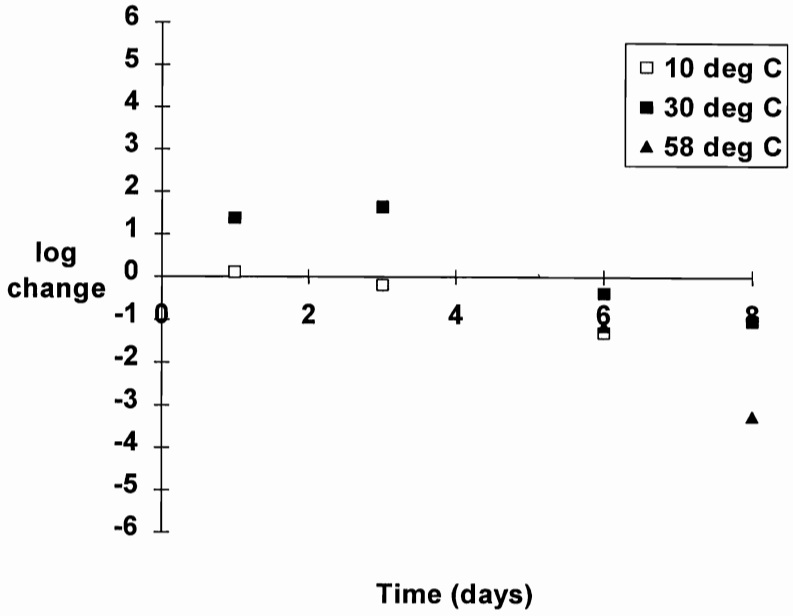
(a) 14% humidity



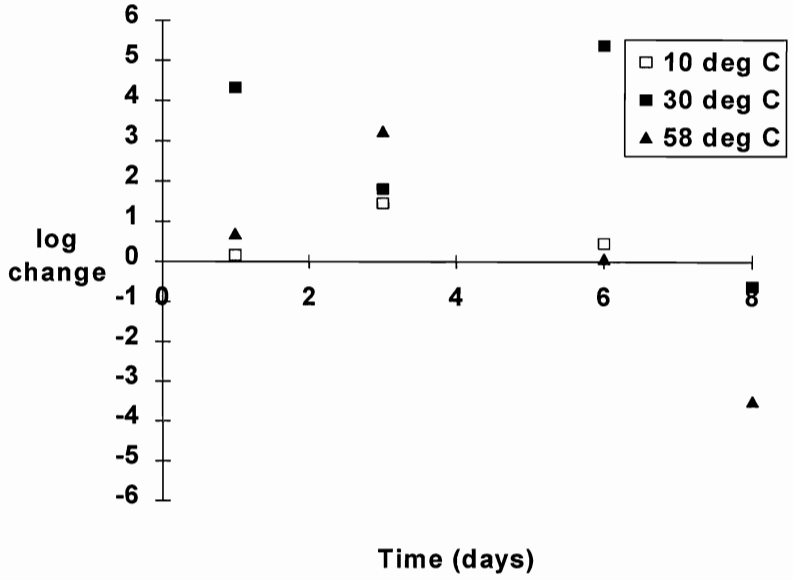
(b) 30% humidity



(c) 75% humidity



(d) 100% humidity



CONCLUSIONS

As cleaning and disinfection are the major controls of surface hygiene, extensive studies of various cleaning parameters were undertaken.

The cleaning studies showed that spraying attached bacteria with water pressures above 250 psi did not improve the removal of the attached organisms. In fact, spraying at pressures above 250psi may exacerbate the dispersal of microorganisms from the surface and the spread of contamination.

Dried-on biofilms were more difficult to remove and therefore surfaces should be cleaned as soon as possible after production finishes.

Increasing the temperature of the water used to spray the surfaces did not increase the removal of attached microorganisms, although higher temperatures (>45°C) reduced the viability of the microorganisms and may therefore reduce the spread of contamination. The fact that hot water is not required for bacterial removal from the surface means that energy/cost savings can be made. However, hot water may be required to achieve effective soil removal, particularly in the case of fatty soils.

The optimum distance of the spray lance from the surface was shown to be 250mm approximately. At lower or higher distances, bacterial removal from the surface was reduced. The cleaning studies also showed that spraying for longer than one second did not increase the removal of bacterial biofilms.

The effect of detergents on the viability of microorganisms was found to be species dependent, although the extent of removal of the organisms from the surface was similar regardless of organism or detergent type. The differences in the effect of detergents on the viability of the two attached microorganisms may relate to differing colonisation mechanisms such as differences in the amount and nature of exopolymers. These observations have relevance for the limitation of the spread of contamination, as a detergent that reduces the viability of the cells means that a smaller proportion of the organisms removed from the surface will be viable. Detergents are developed to meet certain soil removal requirements rather than the removal of bacteria.

Cleaning studies of factory biofilms showed that mechanical/kinetic energy was particularly important for biofilm removal; however, cleaning techniques that employ high mechanical/kinetic energy generally generate aerosols over a considerable distance and the potential spread of contamination by such systems must be considered.

The presence of soil did not affect the ease of removal of freshly developed biofilms, although dried-on organisms were more difficult to remove in the presence of soil in the case of one of the species studied.

The CIP studies showed that bacteria remained on the surface after prolonged cleaning times and high flow velocities and a small proportion of the cells remained viable even at the high temperature and alkaline pH. If conditions are favourable, these cells may grow on the surfaces between cleaning and production. The extent of removal of bacteria from surfaces by cleaning was approximately two log orders. This is a relatively low removal rate considering processes such as disinfection and pasteurisation require a five log reduction in microorganisms, and therefore, because bacteria are difficult to remove from the surfaces, disinfection is required to significantly reduce the viability of the remaining attached population.

The disinfection studies showed that the treatment of attached bacteria and multilayers of organisms with certain detergents prior to disinfection had an additive effect, enhancing the log reductions observed. The mechanism for this action may be by removing exopolymer glycocalyces or by inducing physiological stresses that increase the susceptibility to disinfection. This data would seem to suggest that other parameters of the cleaning programme (e.g. temperature, detergent etc), in this case detergent, can influence the susceptibility to disinfectants.

Increasing the temperature increased the disinfection of attached microorganisms. This observation has two implications. Firstly, the use of certain disinfectants in hot water will enhance disinfectant action; however, the disadvantages of using hot water in terms of energy costs and production of condensation may outweigh any increase in disinfectant action. Secondly, the effectiveness of disinfectants in chilled environments will be reduced, and therefore longer contact times, for example, may be required.

Attachment of bacteria to stainless steel is influenced by the presence of material on the surface, and certain surface treatments actually inhibit attachment.

Humidity significantly affected the survival of bacteria on surfaces, and even after one week, viable cells were detected on the surfaces. If bacteria remain on the surfaces after cleaning, they may survive on the surfaces for prolonged periods in a humid environment and therefore remain as a source of contamination.

FUTURE RESEARCH REQUIREMENTS

Bacteria can attach and grow to significant levels on surfaces in the food processing environment; however, there are a number of problems that require further investigation.

Cleaning with the range of detergents and parameters used can reduce the viability of the attached organisms by up to four log orders. Together with subsequent disinfection this may be a suitable control measure; however, there may be a build up of debris on the surface with time.

In terms of microbial removal, however, the cleaning studies showed that only approximately two log orders were removed by any combination of detergent soak and high pressure rinse. Detergents are developed to meet certain soil removal requirements rather than the removal of bacteria. Further studies are required to investigate and optimise the removal of bacteria by detergents. The work reported in this study showed species differences in terms of the effect of detergents on the viability of the cells. A more detailed examination would provide information to more effectively control the spread of contamination. Previous work at Campden has shown that cleaning generates aerosols containing viable microbes (Holah *et al*, 1993a). Detergents that reduce the viability of the attached population would potentially reduce the viable airborne population.

Although this study has established that bacterial microcolonies and biofilms are found on surfaces, there is little information on the detachment of bacteria from surfaces and the transfer of microbes to the product as it passes across the surface, or transfer via utensils.

Another aspect that has not been extensively studied is the effect of the length of time attached to a surface on the ease of removal and resistance of the attached cells to disinfectants.

This study highlighted the effect of temperature on disinfectant efficiency; however, there is limited information on the efficacy of disinfectants against surface attached microbes at low temperatures.

Finally the use of surface treatments to reduce the surface populations through biocidal action or prevention and minimisation of attachment has received little attention in the food processing industry.

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APPENDIX I

MEDIA

All media were prepared in distilled water and autoclaved at 121°C for 20 minutes unless stated otherwise.

Media	Constituents.
Diluent	0.1% (w/v) Bacteriological peptone. 0.85% (w/v) Sodium chloride.
Inactivator	0.3% (w/v) Soya lecithin. 3.0% (v/v) Tween 80. 0.5% (w/v) Sodium thiosulphate. 0.1% (w/v) L-histidine. 1.0% (v/v) 0.25N phosphate buffer (KH_2PO_4) pH 7.2.
Phosphate buffer	3.4% (w/v) Potassium dihydrogen phosphate.
Growth medium	0.1% (w/v) Bacteriological peptone. 0.07% (w/v) Yeast extract.
SPYE broth	3.0% (w/v) Special peptone. 0.25% (w/v) Yeast extract.
Water of Standard Hardness	Solution A: 3.174% (w/v) anhydrous magnesium chloride and 7.399% (w/v) anhydrous calcium chloride. Solution B: 5.603% (w/v) sodium hydrogen carbonate and sterilise by filtration through a filter with a maximum effective pore size of 0.22μm. Add 0.75ml Solution A and 1ml Solution B to 100ml sterile distilled water.
Bovine Albumin	0.03% (w/v) Bovine albumin.

APPENDIX II

SURVEY OF FOOD INDUSTRY CLEANING PRACTICES

Questionnaires on the cleaning practices used by factories were sent out to Campden member companies. The questionnaires are included in Appendix II. Questionnaires were sent out in 1987 and 1992; however, the 1992 questionnaire was modified to obtain additional and slightly different information to the 1987 survey. The results of both surveys are included to show the similarities and changes in practices over the five year period. There were 61 replies to the 1987 survey and 54 replies to the 1992 survey.

Food Industry Survey 1987 (61 replies)

NUMBER OF FACTORIES HAVING CLEANING MANUALS

	YES	NO
NUMBER PERCENTAGE (%)	49 80	12 20

MICROBIOLOGICAL TESTING

		YES	NO
Routine microbiological test on cleaned surfaces	Number Percentage (%)	40 66	21 34
Routine microbiological test on food contact or environmental surfaces	Number Percentage (%)	43 70	18 30

SAMPLING METHOD

	Number	Percentage (%)
Swab	46	76
Rinsing	7	11
Contact plates	3	5
Visual	1	2
Miscellaneous	4	6

FLOW RATE USED IN CLEANING SYSTEMS

Flow Rate (L/MIN)	Number
315	1
50-40	2
20 - 15	2
15 - 10	7
10 - 8	8
NUMBER THAT SPECIFIED	20

MICROBIOLOGICALLY TESTED AREAS

Area	Number	Percentage (%)
Preparation	12	19
Production	10	15
Preparation/Production	19	29
Post Process	17	26
Environment	6	9
Random	1	2
TOTAL	65	

TYPES OF SPRAY CLEANING USED

Type of Spray Cleaner	Number
Portable Ring main Both Unspecified	22 10 3 1
Total number using spray cleaners	36 (= 59%)

SPRAY PRESSURE USED FOR CLEANING

Pressure (psi)	Number
2500 - 2000	4
1500 - 1000	15
800 - 100	9
100 - 80	1
NUMBER THAT SPECIFIED	19

DISINFECTANT AND SANITIZER USAGE

Product Group	No. of Applications	No. of Brands
ALKALINE	102	67
CHLORINE	76	44
QAC	56	30
ACID	49	31
AMPHOLYTIC	12	8
GENERAL PURPOSE	14	9
HYDROGEN PEROXIDE	3	3
IODINE	1	1
ALKALI	7	7
CATIONIC	2	1
GAS	1	1
GEL	1	1
ENZYME	2	2
DEGREASER	4	3
DESCALER	1	1
AROMATIC SOLVENT	1	1
SOAP	5	5
MIXTURES	3	3
UNCLASSIFIED	34	30
TOTAL	374	248

USE OF DETERGENTS AND SANITIZERS AT DIFFERENT TEMPERATURES IN THE FOOD INDUSTRY

	NUMBERS OF DETERGENT/SANITIZERS USED AT DIFFERENT TEMPERATURES				
PRODUCT GROUP	HOT	COLD	HOT & COLD	WARM	UNSPECIFIED
ALKALINE	67	25	0	7	3
CHLORINE	45	12	14	1	4
QAC	12	36	0	8	0
ACID	23	18	5	1	2
AMPHOTERIC	0	9	2	1	0
GENERAL PURPOSE	7	2	1	4	0
HYDROGEN PEROXIDE	1	2	0	0	0
IODINE	0	1	0	0	0
ALKALI	2	1	2	2	0
CATIONIC	0	2	0	0	0
GAS	0	1	0	0	0
GEL	0	0	0	1	0
ENZYME	1	1	0	0	0
DEGREASER	1	2	0	0	1
DESCALER	1	0	0	0	0
AROMATIC SOLVENT	0	1	0	0	0
SOAP	2	0	0	2	1
MIXTURES	0	2	0	1	0
UNCLASSIFIED	12	18	0	4	0

Food Industry Survey 1992 (54 replies)

NUMBER OF FACTORIES HAVING CLEANING MANUALS

	Number	Percentage (%)
Yes	46	85
No	7	13
Unspecified	1	2

CLEANING RESPONSIBILITY OF STAFF

	Number	Percentage (%)
Factory staff only	40	74
Contract staff only	0	0
Both	14	26

ASSESSMENT OF CLEANING EFFICIENCY

	Number	Percentage (%)
Visually only	12	22
Microbiologically only	3	6
Both	39	72

RECORDING OF VISUAL ASSESSMENT

	Number	Percentage (%)
Yes	35	65
No	18	33
Unspecified	1	2

MICROBIOLOGICALLY TESTED AREAS

	Number	Percentage (%)
Preparation only	0	
Preparation and production only	7	13
Preparation, production and post process only	6	11
Preparation, production, post process and high risk	13	24
Preparation, post process and high risk only	1	2
Production and post process only	8	15
Production, post process and high risk only	4	7
Post process only	1	2
Post process and high risk only	1	2
High risk only	1	2
None	12	22

TIME OF MICROBIAL ASSESSMENT

	Number	Percentage (%)
Before cleaning only	0	0
After cleaning only	10	30
Before and after cleaning	3	9
During production only	1	3
Before and after cleaning and during production	8	24
After cleaning and during production	10	30
As required	1	3

MICROBIAL SAMPLING METHOD

	Number	Percentage (%)
Swab	24	57
Contact plate	5	12
Swab and contact plate	3	7
Swab and rinse samples	7	17
Swab, contact plate and rinse sample	3	7

MICROBIOLOGICAL TESTING OF SURFACES

	Number	Percentage (%)
Equipment/food contact only	28	67
Factory surfaces only	1	2
Equipment/food contact and factory surfaces	13	31

MICROORGANISMS TESTED FOR

Test microorganisms	Number	Percentage (%)
Total viable count	11	26
Total viable count and coliforms	5	12
Total viable count, coliforms and Enterobacteriaceae	2	5
Total viable count, coliforms, Enterobacteriaceae and yeasts/moulds	7	17
Total viable counts, coliforms and yeasts/moulds	9	21
Total viable count and Enterobacteriaceae	2	5
Total viable count, Enterobacteriaceae and yeasts/moulds	2	5
Total viable counts and yeasts/moulds	1	2
Coliforms and Enterobacteriaceae	1	2
Yeasts/moulds	2	5

OTHER SPECIFIC MICROORGANISMS TESTED FOR

Microorganism	Number	Percentage (%)
Salmonella	7	26
Listeria	5	19
Staphylococcus aureus	5	19
Clostridia	2	7
Streptococcus faecalis	2	7
Bacillus cereus	1	4
Lactobacillus	1	4
Acetic acid bacteria	1	4
Spores	1	4
Thermophiles	1	4
Anaerobes	1	4

USAGE OF RAPID METHODS TO ASSESS SURFACE CLEANNESS

	Number	Percentage (%)
Yes	11	20
No	37	69
Unspecified	6	11

TYPE OF RAPID METHOD USED

	Number
ATP	10
ELISA	1

QUALITY ASSURANCE OR HACCP PROCEDURE TO MONITOR PERFORMANCE OF SANITATION PROCEDURE

	Number	Percentage (%)
Yes	27	50
No	23	44
Unspecified	4	7

WATER HARDNESS

	Number	Percentage (%)
Hard	29	54
Soft	19	35
Hard and soft	4	7
Unspecified	2	4

USAGE OF HIGH PRESSURE CLEANING SYSTEMS

	Number	Percentage (%)
Yes	39	72
No	14	26
Unspecified	1	2

TYPE OF HIGH PRESSURE CLEANING SYSTEM USED

	Number	Percentage (%)
Portable system	13	33
Ring main	17	44
Both	9	23

WATER AND DETERGENT USAGE OF HIGH PRESSURE CLEANING SYSTEMS

	Number	Percentage (%)
Cold water	4	10
Cold water and detergent/disinfectant	6	15
Hot water	12	31
Hot water and detergent/disinfectant	7	18
Cold water, hot water and detergent/disinfectant	10	26

Summary of food industry surveys

The results of the surveys show that over the period from 1987 to 1992 the percentage of factories having cleaning manuals increased from 80 to 85%. The 1992 questionnaire included questions about cleaning responsibility and this showed that in the majority of factories (76%) cleaning was the responsibility of the factory staff; however, in 26% of factories contract cleaning staff were also involved. The use of contract cleaners in the UK remains low compared to that in other countries, particularly the USA.

Microbiological sampling and testing was investigated in both surveys. The 1987 survey found that 87% of factories used swab and rinse sampling methods and 5% used contact plates; however, the 1992 survey found that 74% of factories used swabs and rinse sampling methods and the percentage using contact plates had increased to 12%. This may be due to the limited laboratory facilities required to undertake sampling by contact plates.

The 1987 survey found that 66% of factories routinely performed microbiological analysis of cleaned surfaces, and 70% of factories routinely performed analysis on food contact or environmental surfaces. The 1992 survey found that 67% of factories sampled equipment and food contact surfaces, whilst 31% of factories sampled equipment and food contact surfaces and factory environmental surfaces.

The 1992 survey produced information about the timing of microbial assessment and showed that 84% of factories performed microbiological assessments after cleaning, 30% of the factories only sampled after cleaning, 54% of factories sampled both during production and after cleaning, and 24% of factories performed microbiological assessment of the surfaces before and after cleaning and during production. It is interesting to note the increased usage of rapid techniques; in 1987 only 6% of factories used techniques other than the traditional methods; however, by 1992 19% of factories were using ATP bioluminescence to monitor hygiene.

The surveys highlight a number of trends in cleaning practices. For example, in 1987, 61% of factories using spray cleaning used portable systems, and 27% used ring main systems, but in 1992 the portable system usage had reduced to 33% and the ring main usage had increased to 44%. This may be due to relative changes in costs of the two systems.

The types of chemicals used for disinfection purposes in descending order of usage were alkaline, chlorine, quaternary ammonium compounds (QAC), acid and ampholytic. This order of usage was also reported for cleaning chemicals. In the 1987 survey 66% of uses of alkalines were in hot water, and 59% of chlorine uses were in hot water. Replies to the 1992 survey showed that 19% used hot water and detergent/disinfectant and 26% used cold water, hot water and detergent disinfectant. Overall 75% of factories used hot water at some stage in their cleaning regime.

The usage of high pressures for spray washing appeared to be similar in 1987 (78% approximately) and 1992 (72%) despite increased awareness of the problems of aerosol generation and potential contamination risks.

The 1992 survey highlighted that 50% of factories were using Quality Assurance or HACCP procedures to monitor the performance of the sanitation procedure.

The information from the 1987 and 1992 surveys was used to select the spray pressures, chemical types, flow rates and temperatures in the cleaning studies described in Section 3 which examined the effect these parameters had on the removal of bacterial biofilms.

APPENDIX III

QUESTIONNAIRES USED IN THE CLEANING PRACTICES SURVEY

Company _____

Location _____

SECTIONS 1 - 5

DETERGENT/SANITISER AND APPLICATION

Please read attached notes before completing sections.

Detergent/Sanitiser used and Type (1)	Application (2)				Equipment /Surface (3)	Surface Material (4)	Product (5)	Special Comments
	Temp.	Conc.	Method	Frequency				

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SECTION 6

Is the water at the factory considered to be Hard/Soft ?

SECTION 7

Do you have a cleaning procedure manual ? Yes/No

SECTION 8 & 9

8. Are routine microbiological tests carried out on cleaned surfaces ? Yes/No

9. Are routine microbiological tests carried out on food contact or environmental surfaces. Yes/No

If you answered yes to either section 8 or 9 please complete the table below.

Area	Sampling Stage	Sampling Method	Type of Count	Media	Incubation Time/Temp.	Satisfactory Final Counts

SECTION 10

Spray cleaning.

If you use high pressure cleaning systems, please specify:

Pressure at nozzle.

Flow rate

Ring main or portable unit

Manufacturer

SECTION 11

Research needs.

Listed below are some of the activities currently being undertaken at Campden. Please rank these activities in order of their importance to fulfill your requirements. Indicate the most important by number 1 and the least by number 8.

Research Activity	Ranking
1. Determination of the cleanability of various materials/ surfaces.	
2. Investigation of the nature of surface soils to be cleaned.	
3. Rapid methodology (<1 hour) to determine when to clean.	
4. Rapid methodology (<1 hour) to determine surface microbiological cleanliness after cleaning.	
5. Information on required spray pressures and temperatures.	
6. Information on selection of detergent/sanitiser and in use concentrations.	
7. The development of hygienic training/educational material (Video's etc.)	
8. Cleaning procedures (design and implementation of sanitation programmes).	

Other requirements: please specify

Thank you for your co-operation.

Signed: Position:

Company:

QUESTIONNAIRE NOTES

SECTION 1

If known please include chemical company, brand name and type. Type refers to QAC, acid, alkaline, chlorine, amphoteric etc. For example: Reddish Savilles, Balmoral LMS, QAC.

If you make your own mixtures of detergent/sanitisers give details of the materials used and their proportions.

If no chemicals are used and the equipment/surface is just hosed down, enter "hosed down only" and complete the other columns.

SECTION 2

Temperature: If temperature is not known, state whether ambient, warm or hot.

Concentration: Indicate the normal in use concentration.

Method: Describe as manual, low/high pressure, CIP, foam etc.

Frequency: Please fill this column in with a letter representing the frequency shown in the table below.

Frequency	Letter
At the end of a production run.	A
Once a day	B
More than once a day	C
Once a week	D
Once a month	E
When thought necessary	F
Other (please state)	

SECTION 3

We are interested to obtain information about the total cleaning of a work place. Could you therefore include in this section - tank, inspection belt, floor, wall, ceiling, elevator etc.

SECTION 4

Please state the surface to be cleaned, e.g. stainless steel, mild steel, polypropylene, high density nylon, concrete etc.

SECTION 5

If surface cleaned is in contact with food please state food type, e.g. meat, vegetable, dairy, recipe pack etc.

SPECIAL COMMENTS

Describe anything else that is required in the cleaning programme, e.g. descaling, degreasing etc.

SECTION 8 and 9

Notes for completion of table.

Area:	State preparation, post process or environment.
Sampling stage:	State routine, pre-cleaning or post-cleaning.
Sampling method:	Indicate sampling technique, e.g. swabbing, rinsing contact plates, DEFT etc.
Satisfactory final count:	If you have bacteriological specifications to be met, please state range of satisfactory counts e.g. less than 500 per 4 square inches.

LRK/SCM/436

FOOD INDUSTRY SURVEY 1992

Company: _____

Location: _____

SECTION A

Please tick appropriate boxes or complete the blank spaces

1. Do you have a sanitation programme/cleaning procedure manual?
   Yes ☐
   No ☐

2. Is cleaning done by your staff? ☐
   contract staff? ☐
   both? ☐

3. Is cleaning efficiency assessed visually? ☐ (see 4)
   microbiologically? ☐ (see 5)
   other means? _____
   _____

4. Is the visual assessment recorded?
   Yes ☐
   No ☐

5. (i) Which areas are microbiologically tested?
   Preparation? ☐
   Production? ☐
   Post process? ☐
   High Risk? ☐
   Others? _____
   _____

(ii) Is microbiological testing done before cleaning? ☐
after cleaning? ☐
during production? ☐
other? _____
_____

(iii) What sampling method is used?
Swab ☐
Contact plate ☐
Rinse ☐
Others _____
_____

(iv) What surfaces are tested?

_____

_____

(v) What microorganisms are tested for and what is considered a satisfactory final count?

Microorganism		Satisfactory final count (please specify area size)
Total viable count	☐	_____
Coliform	☐	_____
Enterobacteriaceae	☐	_____
Yeast/moulds	☐	_____
Others	_____	_____
	_____	_____

6. (i) Are rapid methods used to assess surface cleanness? Yes ☐ No ☐

(ii) Method To detect (soil, microorganisms, etc - please specify).

ATP ☐ _____
DEFT ☐ _____
Others ☐ _____
_____

7. Do you have a structured Quality Assurance or HACCP procedure to monitor the performance of the sanitation programme? Yes ☐ No ☐

8. Is the water at the factory - hard? ☐
- soft? ☐

9. Spray cleaning systems.

Do you use a high pressure cleaning system? Yes ☐ No ☐

Is the system a portable system? ☐
a ring main? ☐

Manufacturer _____

Does the system use cold water? ☐
hot water? ☐
detergent/disinfectant mixes (please specify). _____
_____

What is the pressure at the nozzle? _____

SECTION B - DETERGENT/DISINFECTANT USAGE

Please tick appropriate boxes or complete blank spaces. Use one page for each product

Manufacturer _____

Brand Name _____

[1]Detergent/disinfectant type _____

Food product _____

AREA USED IN	PREPARATION		PRODUCTION		POST-PROCESS	
SURFACE USED ON	[2]EQUIPMENT	[3]FACTORY	EQUIPMENT	FACTORY	EQUIPMENT	FACTORY
HIGH RISK AREA	☐	☐	☐	☐	☐	☐
LOW RISK AREA	☐	☐	☐	☐	☐	☐
CONCENTRATION USED AT	____	____	____	____	____	____
TEMPERATURE USED AT	____	____	____	____	____	____
APPLICATION METHOD						
CIP	☐	☐	☐	☐	☐	☐
MANUAL	☐	☐	☐	☐	☐	☐
HIGH PRESSURE	☐	☐	☐	☐	☐	☐
LOW PRESSURE	☐	☐	☐	☐	☐	☐
MIST SPRAY	☐	☐	☐	☐	☐	☐
FOAM	☐	☐	☐	☐	☐	☐
GEL	☐	☐	☐	☐	☐	☐
OTHER (SPECIFY)	____	____	____	____	____	____
FREQUENCY OF APPLICATION						
DURING PRODUCTION	☐	☐	☐	☐	☐	☐
AFTER PRODUCTION	☐	☐	☐	☐	☐	☐
DAILY	☐	☐	☐	☐	☐	☐
ONCE A WEEK	☐	☐	☐	☐	☐	☐
ONCE A MONTH	☐	☐	☐	☐	☐	☐
OTHER (SPECIFY)	____	____	____	____	____	____

1 Detergent/disinfectant type - specify if product is a chlorine, alkaline, acid, QAC, amphoteric etc. substance
2 Equipment - product contact equipment
3 Factory - non product contact, e.g. walls, floors, drains

Manufacturer _____

Brand Name _____

[1]Detergent/disinfectant type _____

Food product _____

AREA USED IN	PREPARATION		PRODUCTION		POST-PROCESS	
SURFACE USED ON	[2]EQUIPMENT	[3]FACTORY	EQUIPMENT	FACTORY	EQUIPMENT	FACTORY
HIGH RISK AREA	☐	☐	☐	☐	☐	☐
LOW RISK AREA	☐	☐	☐	☐	☐	☐
CONCENTRATION USED AT	_____	_____	_____	_____	_____	_____
TEMPERATURE USED AT	_____	_____	_____	_____	_____	_____
APPLICATION METHOD						
CIP	☐	☐	☐	☐	☐	☐
MANUAL	☐	☐	☐	☐	☐	☐
HIGH PRESSURE	☐	☐	☐	☐	☐	☐
LOW PRESSURE	☐	☐	☐	☐	☐	☐
MIST SPRAY	☐	☐	☐	☐	☐	☐
FOAM	☐	☐	☐	☐	☐	☐
GEL	☐	☐	☐	☐	☐	☐
OTHER (SPECIFY)	_____	_____	_____	_____	_____	_____
FREQUENCY OF APPLICATION						
DURING PRODUCTION	☐	☐	☐	☐	☐	☐
AFTER PRODUCTION	☐	☐	☐	☐	☐	☐
DAILY	☐	☐	☐	☐	☐	☐
ONCE A WEEK	☐	☐	☐	☐	☐	☐
ONCE A MONTH	☐	☐	☐	☐	☐	☐
OTHER (SPECIFY)	_____	_____	_____	_____	_____	_____

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Manufacturer _____

Brand Name _____

[1]Detergent/disinfectant type _____

Food product _____

AREA USED IN	PREPARATION		PRODUCTION		POST-PROCESS	
SURFACE USED ON	[2]EQUIPMENT	[3]FACTORY	EQUIPMENT	FACTORY	EQUIPMENT	FACTORY
HIGH RISK AREA	☐	☐	☐	☐	☐	☐
LOW RISK AREA	☐	☐	☐	☐	☐	☐
CONCENTRATION USED AT	____	____	____	____	____	____
TEMPERATURE USED AT	____	____	____	____	____	____
APPLICATION METHOD						
CIP	☐	☐	☐	☐	☐	☐
MANUAL	☐	☐	☐	☐	☐	☐
HIGH PRESSURE	☐	☐	☐	☐	☐	☐
LOW PRESSURE	☐	☐	☐	☐	☐	☐
MIST SPRAY	☐	☐	☐	☐	☐	☐
FOAM	☐	☐	☐	☐	☐	☐
GEL	☐	☐	☐	☐	☐	☐
OTHER (SPECIFY)	____	____	____	____	____	____
FREQUENCY OF APPLICATION						
DURING PRODUCTION	☐	☐	☐	☐	☐	☐
AFTER PRODUCTION	☐	☐	☐	☐	☐	☐
DAILY	☐	☐	☐	☐	☐	☐
ONCE A WEEK	☐	☐	☐	☐	☐	☐
ONCE A MONTH	☐	☐	☐	☐	☐	☐
OTHER (SPECIFY)	____	____	____	____	____	____

[1] Detergent/disinfectant type - specify if product is a chlorine, alkaline, acid, QAC, amphoteric etc. substance
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Manufacturer

Brand Name _____

[1]Detergent/disinfectant type _____

Food product _____

AREA USED IN	PREPARATION		PRODUCTION		POST-PROCESS	
SURFACE USED ON	[2]EQUIPMENT	[3]FACTORY	EQUIPMENT	FACTORY	EQUIPMENT	FACTORY
HIGH RISK AREA	☐	☐	☐	☐	☐	☐
LOW RISK AREA	☐	☐	☐	☐	☐	☐
CONCENTRATION USED AT	_____	_____	_____	_____	_____	_____
TEMPERATURE USED AT	_____	_____	_____	_____	_____	_____
APPLICATION METHOD						
CIP	☐	☐	☐	☐	☐	☐
MANUAL	☐	☐	☐	☐	☐	☐
HIGH PRESSURE	☐	☐	☐	☐	☐	☐
LOW PRESSURE	☐	☐	☐	☐	☐	☐
MIST SPRAY	☐	☐	☐	☐	☐	☐
FOAM	☐	☐	☐	☐	☐	☐
GEL	☐	☐	☐	☐	☐	☐
OTHER (SPECIFY)	_____	_____	_____	_____	_____	_____
FREQUENCY OF APPLICATION						
DURING PRODUCTION	☐	☐	☐	☐	☐	☐
AFTER PRODUCTION	☐	☐	☐	☐	☐	☐
DAILY	☐	☐	☐	☐	☐	☐
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ONCE A MONTH	☐	☐	☐	☐	☐	☐
OTHER (SPECIFY)	_____	_____	_____	_____	_____	_____

[1] Detergent/disinfectant type - specify if product is a chlorine, alkaline, acid, QAC, amphoteric etc. substance
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Manufacturer _____

Brand Name _____

[1]Detergent/disinfectant type _____

Food product _____

AREA USED IN	PREPARATION		PRODUCTION		POST-PROCESS	
SURFACE USED ON	[2]EQUIPMENT	[3]FACTORY	EQUIPMENT	FACTORY	EQUIPMENT	FACTORY
HIGH RISK AREA	☐	☐	☐	☐	☐	☐
LOW RISK AREA	☐	☐	☐	☐	☐	☐
CONCENTRATION USED AT	____	____	____	____	____	____
TEMPERATURE USED AT	____	____	____	____	____	____
APPLICATION METHOD						
CIP	☐	☐	☐	☐	☐	☐
MANUAL	☐	☐	☐	☐	☐	☐
HIGH PRESSURE	☐	☐	☐	☐	☐	☐
LOW PRESSURE	☐	☐	☐	☐	☐	☐
MIST SPRAY	☐	☐	☐	☐	☐	☐
FOAM	☐	☐	☐	☐	☐	☐
GEL	☐	☐	☐	☐	☐	☐
OTHER (SPECIFY)	____	____	____	____	____	____
FREQUENCY OF APPLICATION						
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AFTER PRODUCTION	☐	☐	☐	☐	☐	☐
DAILY	☐	☐	☐	☐	☐	☐
ONCE A WEEK	☐	☐	☐	☐	☐	☐
ONCE A MONTH	☐	☐	☐	☐	☐	☐
OTHER (SPECIFY)	____	____	____	____	____	____

[1] Detergent/disinfectant type - specify if product is a chlorine, alkaline, acid, QAC, amphoteric etc. substance
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Manufacturer

Brand Name _____

[1]Detergent/disinfectant type _____

Food product _____

AREA USED IN	PREPARATION		PRODUCTION		POST-PROCESS	
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HIGH RISK AREA	☐	☐	☐	☐	☐	☐
LOW RISK AREA	☐	☐	☐	☐	☐	☐
CONCENTRATION USED AT	_____	_____	_____	_____	_____	_____
TEMPERATURE USED AT	_____	_____	_____	_____	_____	_____
APPLICATION METHOD						
CIP	☐	☐	☐	☐	☐	☐
MANUAL	☐	☐	☐	☐	☐	☐
HIGH PRESSURE	☐	☐	☐	☐	☐	☐
LOW PRESSURE	☐	☐	☐	☐	☐	☐
MIST SPRAY	☐	☐	☐	☐	☐	☐
FOAM	☐	☐	☐	☐	☐	☐
GEL	☐	☐	☐	☐	☐	☐
OTHER (SPECIFY)	_____	_____	_____	_____	_____	_____
FREQUENCY OF APPLICATION						
DURING PRODUCTION	☐	☐	☐	☐	☐	☐
AFTER PRODUCTION	☐	☐	☐	☐	☐	☐
DAILY	☐	☐	☐	☐	☐	☐
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OTHER (SPECIFY)	_____	_____	_____	_____	_____	_____

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Signed: _____ Position: _____

Thank you for your cooperation