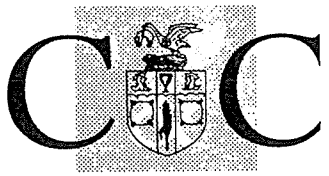

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Biofilms and their Detection in the Food Industry

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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 the product by vectors such as personnel, pests, air movement or cleaning regimes.

The aim of this work was to identify food processing environments which allow biofilm development to occur. A range of factory environments were studied to cover a number of product types and types of processing. Biofilm development was associated with the presence of water, moisture or condensation. Biofilms were particularly associated with poor practices, such as inadequate ventilation resulting in condensation, and poor equipment design features.

Biofilm development was found to be time dependent; bacterial microcolonies may develop into biofilms given sufficient time and nutrients. The most common species found on the surfaces were pseudomonads and similar bacteria, coliforms and staphylococci. The presence of staphylococci, which are particularly associated with the skin, highlights the importance of correctly worn protective clothing and effective hand washing procedures.

INTRODUCTION

A biofilm consists of microbes and a matrix of extracellular products in association with a surface. 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 reduce the level of 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 completely remove the 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 1). 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.

The aim of this work was to identify food processing environments which allow biofilm development to occur. A range of different food processing environments were examined for the presence of significant levels of microorganisms on food contact and non-food contact surfaces. The levels and types of microorganisms present were determined.

The results of other studies to investigate the efficacy of cleaning techniques in terms of bacterial biofilm removal are described by Gibson *et al* (1995).

TABLE 1

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 (inoculated)	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

METHODS

Selection of Factory Sites and Factory Work Programme

A range of factories were selected from Campden members willing to allow site visits to cover a range of product types (salad, meat, fish, poultry and dairy) and types of processing (freezing, canning, chilling and baking).

As there is little information about the importance of biofilms in the food processing environment, the objective of the factory visits was to investigate biofilm development in order to elucidate factors promoting surface growth, levels of microbes in a variety of locations, and types of microorganisms present, and test cleaning efficiency where possible. The actual work programme conducted at each site was dependent on a number of factors including the availability of attachment sites for stainless steel coupons, production and cleaning schedules, and the presence of environments expected to promote biofilm development.

Swabbing

Surfaces were swabbed with cotton swabs (Sterilin) pre-moistened with diluent (see Appendix I), and placed in a 10ml volume consisting of 9ml diluent and 1ml inactivator (see Appendix I). Routinely 25cm^2 was sampled, and where comparisons were made, for example before and after cleaning, adjacent surfaces were sampled. Swabs were transported to the laboratory in a cool box containing ice packs. 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.

The swabbing technique was also used to sample the population attached to test surfaces in the laboratory.

Attachment of Stainless Steel Coupons

Stainless steel plates (100 x 40mm) with a 2B finish were firmly attached in a variety of positions in a range of food processing environments using clamps (Holah *et al*, 1989). These positions included areas adjacent to the main product flow, yet still receiving product contact through splashing, and a variety of environmental locations. After various contact times the plates were then removed and prepared for examination by Direct Epifluorescent Microscopy.

Total Viable Count (TVC) Determination

The resuspension fluid (from the swab) was serially diluted in diluent (see Appendix I) and duplicate 1ml samples were removed for pour plating using Nutrient Agar (Oxoid). Plates were incubated at 30°C for 2 days.

Identification of Isolates

Several of the commonest colony types from the TVC plates were streaked for purity on Nutrient Agar. Preliminary identification involved examination of Gram reaction and catalase, oxidase and coagulase tests (where appropriate). The Vitek computerized identification system was used to identify the isolates to species level (Vitek Systems, 595 Anglum Drive, Hazelwood, Missouri, USA).

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 epifluoresence 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 coverage was converted to counts/cm^2 using a calibration based on measuring the size of twenty individual organisms.

ATP Bioluminescence

ATP bioluminescence was used to evaluate the effectiveness of factory cleaning regimes. The ATP system detects ATP from microbial cells and product debris, and therefore is an effective measure of the efficacy of the cleaning regime which should eliminate both product and microbial cells.

Surfaces ($25cm^2$) were sampled by swabbing with ATP-free swabs pre-moistened in swab diluent (Total Hygiene Monitoring kit, Biotrace). The swab was resuspended in 800ml swab diluent, 200μl was transferred to a clean cuvette and 100μl of enzyme (reconstituted according to manufacturer's instructions) added and the light output read immediately in the Multi-Lite luminometer (Biotrace).

The following controls were also conducted:

Machine blank - an empty cuvette placed in the luminometer.

Swab control - sterile swab resuspended in diluent and treated as described above.

Reagent control - 100μl enzyme added to 200μl swab diluent.

RESULTS AND DISCUSSION

The table below summarises the factories visited, and lists the type of products each produced.

TABLE 2

SUMMARY OF THE FACTORIES EXAMINED FOR BIOFILM DEVELOPMENT

Factory Number	Products
1	Canned products
2	Canned products
3	Fresh processed meats
4	Poultry
5	Meat, pastry, pizza
6	Pizza, fried products
7	Pizza
8	Fish cakes
9	Fish - Fillets and cakes
10	Protein substitute
11	Cheese
12	Vegetable and salad products
13	Vegetable and salad products
14	Frozen peas
15	Biscuits
16	Biscuits
17	Spices, flavourings

Individual Factory Data

FACTORY 1

This factory produced a variety of canned products. Table 3 shows the surface microbial levels in a variety of locations. The microbial counts on the bean filler stand which was continually exposed to product were high. However, beans picking up such contamination would receive further processing during canning. Control of microbial contamination post process is critical in order to prevent can leaker spoilage. The counts along the discharge lines were low. Significant levels of microorganisms were found on the discard chute, where moisture from the can line trickled over the surface.

The bean blancher and extractor system showed some interesting results. Although relatively low levels were present on the blancher itself, the steam exit chimney had significantly higher levels of microbial contamination. This is a very humid and warm (27°C) environment and therefore microbial proliferation occurred. The blancher extractor system was sampled by swabbing and attaching stainless steel coupons for various time intervals. Table 4 shows the levels of microorganisms detected by DEM. Biofilms were detected in the waste can area after 24 hours and in the extractor system. Table 5 shows the microorganisms identified from a variety of locations. Figure 1 shows the blancher extractor system and Figure 2 shows the position of the stainless steel coupons. Figure 3 shows the development of the biofilm on the stainless steel coupons over the five day period and shows that microcolonies develop into extensive biofilms given sufficient time. The area coverage reached 98% within 120 hours. Figure 4 shows the change in the distribution of ATP levels before and after cleaning of the post process canning lines. Before cleaning 27.3% of surfaces sampled fell in the >50,000 rlu class interval and 81.9% of surfaces sampled gave ATP readings in excess of 150 rlu; however, after cleaning and disinfection 70.6% of surface sampled gave ATP readings of less than 50rlu and 86.2% of surfaces gave ATP readings of less than 150rlu. The ATP results therefore show the efficiency of the cleaning regime.

TABLE 3 - SURFACE MICROBIAL LEVELS

Sample Site	Count/cm²		
	Visit 1	Visit 2	
	TVC NA/30°C	TVC NA/30°C	Thermophiles NA/58°C
Bean filler stand	$1.6x10^7$	-	-
Cooker-cooler discharge	$2.0x10^1$	-	-
	$1.0x10^1$		
Waste can area	$4.4x10^6$	$4.8x10^6$	$1.0x10^2$
Chute in waste can area	-	$5.1x10^6$	$5.1x10^2$
Blancher	-	$1.8x10^1$	$2.7x10^2$
Blancher steam exit	-	$5.1x10^4$	$2.8x10^0$
Blancher extractor	$7.8x10^6$	$1.3x10^6$	$1.3x10^2$

TABLE 4 - BIOFILM DEVELOPMENT ON SURFACES

Coupon Position	Time	DEM Count cells/cm²	% Area Covered	Biofilm Development
Bean filler stand	24h	1.3×10^5	0.2	x
Waste can area	4h	1.7×10^6	0.7	x
	24h	$\geq 3.5 \times 10^7$	15.0	✓
Blancher extractor	24h	$\geq 4.7 \times 10^7$	26.5	✓
28°C	24h	$\geq 4.2 \times 10^7$	23.8	✓
	24h	-	20.2	✓
	48h	-	66.3	✓
	72h	-	85.3	✓
	120h	-	98.4	✓

(-) Sample absent

TABLE 5 - MICROBIAL IDENTIFICATION OF BIOFILM ORGANISMS

Area	Microorganism
Waste can area	*Pseudomonas acidovorans*
	Bacillus (asaccharolytic)
	Pseudomonas paucimobilis
Blancher extractor	*Pseudomonas paucimobilis*
	Flavobacterium indologines
	Pseudomons vesicularis
	Pasteurella haemolytica
	Comamonas acidovorons
	Bacillus sp
	Pseudomonas pickettii
	Flavobacterium sp
	Vibrio alginolyticus
	Sphigobacterium multivorum (Flavobacterium multivorum)
Bean filler stand	*Yersinia pseudotuberculosis*
	Agrobacterium tumefacians
	Escherichia coli
	Enterobacter agglomerans

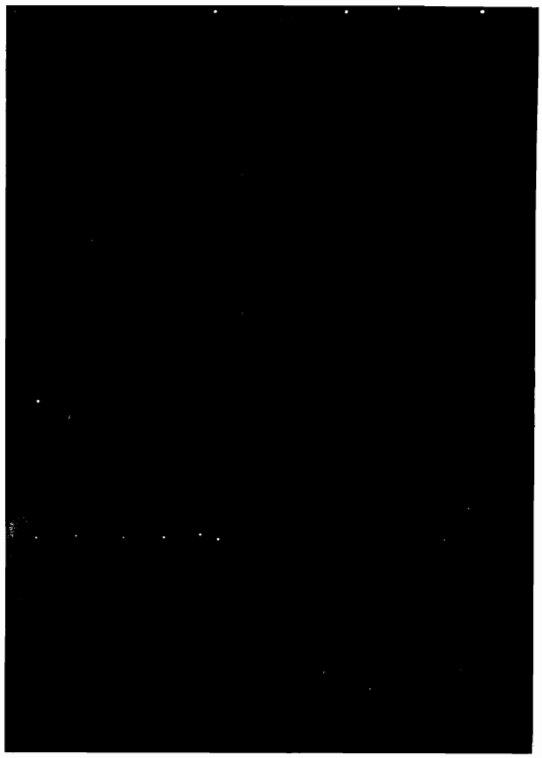
Figure 1 Blancher extractor system of factory 1

Figure 2 Inspection hatch of the extractor system showing the position of the stainless steel coupons

Figure 3 Acridine orange stained coupons removed from the extractor system after (a) 1 day (b) 2 days (c) 3 days and (d) 5 days

(a)

(b)



(c)

(d)

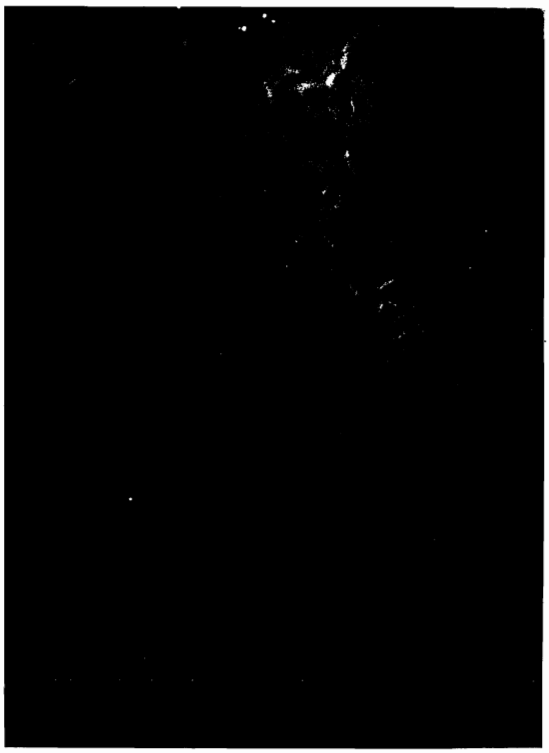
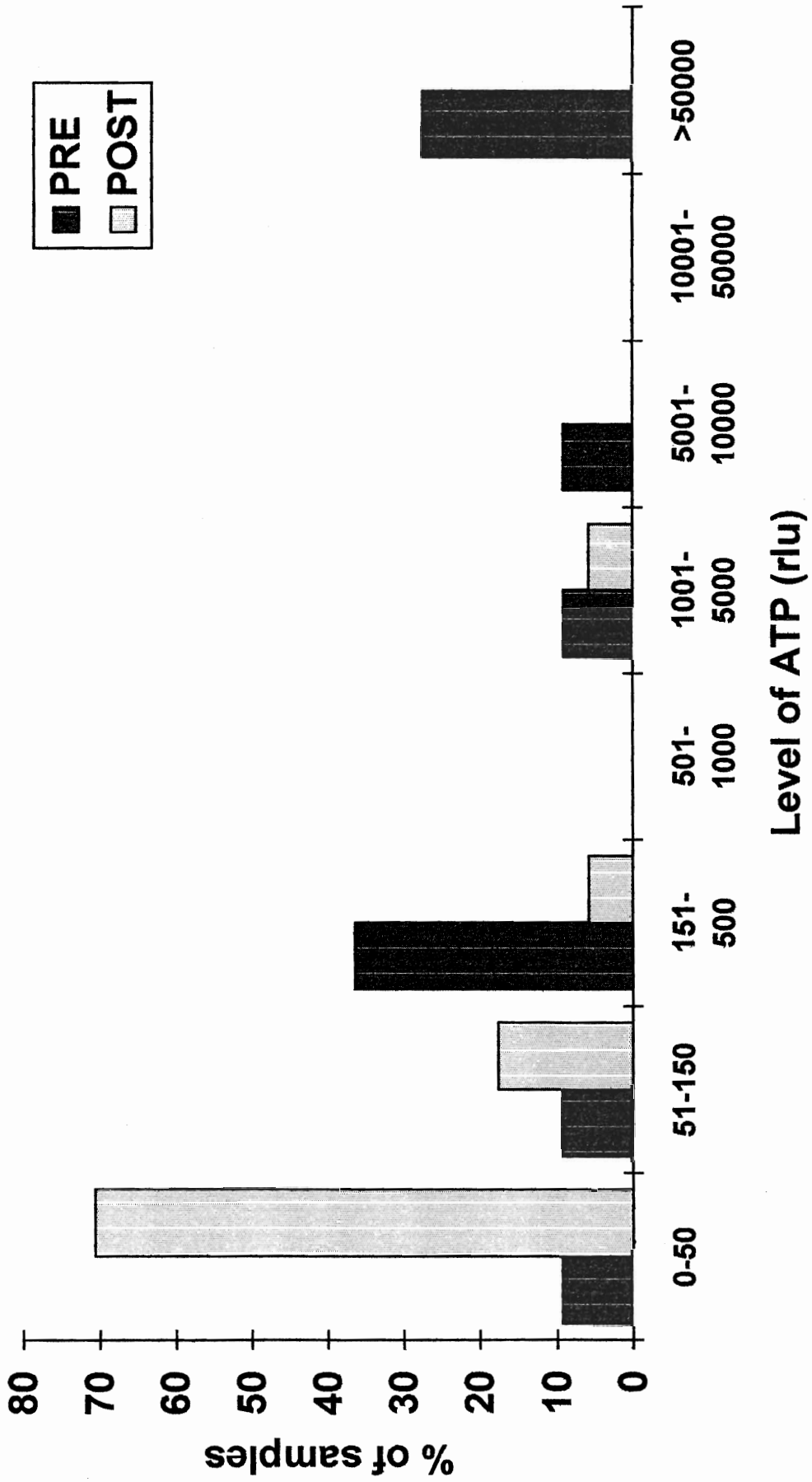


Figure 4 Frequency histogram showing levels of ATP present on the surfaces of the post processing canning lines pre and post cleaning and disinfection at Factory 1



FACTORY 2

Factory 2 produced canned foods. Table 6 shows the surface microbial levels as determined by swabbing. In the vegetable preparation areas, the stainless steel surfaces adjacent to the diced potato and carrot transfer lines showed high levels of microbes. After cooking, the microbial counts on the sides of the potato conveyor were substantially lower; however, the carrot conveyor had high numbers. The microbial levels on the inner surface of the 16oz discharge chimney were quite high for a processed can line, and may result in leaker spoilage of cans. This level exceeds that of 500 per 4 sq. in. (20 cfu/cm^2) as recommended by Thorpe and Everton (1968).

The assessment of biofilm development using stainless steel plates (Table 7) shows that after various time intervals 10^4 cells/cm^2 were detected, and did not constitute a biofilm.

TABLE 6 - SURFACE MICROBIAL LEVELS AS DETERMINED BY SWABBING AND PLATE COUNTS

Sample Site	Total Viable Count (cfu/cm²)
Vegetable preparation:	
After dicing	
Carrot line	7.2×10^4
Potato line	1.5×10^5
After cooking:	
Carrot line	1.8×10^5
Potato line	3.1×10^1
16oz can cooker cooler discharge line:	
Inside chimney	3.7×10^3
Cross bar above cans	$<0.2 \times 10^0$
Beneath line	5.5×10^1
8oz can cooker cooler discharge line:	
Inside chimney	1.8×10^0
Plate above can line	$<0.2 \times 10^0$
Cross bar above cans	1.9×10^1

TABLE 7 - BIOFILM DEVELOPMENT ON STAINLESS STEEL PLATES

Coupon Position	Time (hr)	TVC Count/cm²	Biofilm
Vegetable preparation:			
Carrot line	5	9.6×10^4	x
Potato line	5	5.4×10^4	x
After cooking:			
Carrot line	6	3.6×10^4	x
Potato line	6	2.5×10^4	x
16oz can cooker cooler discharge line:			
Plate above cans	6	8.4×10^4	x
Cross bar above cans	16	4.4×10^4	x
Beneath line	20	1.5×10^5	x
8oz can cooker cooler discharge line:			
Plate above can line	6	1.6×10^4	x
Plate above can line	16	6.7×10^4	x
Cross bar above cans	20	1.7×10^4	x

FACTORY 3

Factory 3 produced fresh and processed meat products. The three different areas at this site were cleaned by different methods: high pressure hot water (65-70°C); foam; and detergent clean followed by disinfection by contract cleaners. Tables 8 and 9 show the efficiency of these methods.

Table 8 shows that the contract cleaners effectively cleaned the inside and outside of the meat filling machine as measured by both TVC and ATP analysis. The levels on the conveyor system were not as effectively reduced, as 7.0×10^2 cfu/cm² remained after cleaning. This probably relates to the difficulties of cleaning conveyor belting; although the ATP results indicate that product debris was effectively removed, the microbial counts were not as effectively reduced.

Table 9 shows that microbial contamination of the order of 10^3 per cm² remained even after cleaning with foam; the ATP levels were also quite high, suggesting inefficient cleaning.

Table 10 shows the levels of contamination after the high pressure hot water wash. The ATP and TVC results were both high even after cleaning. Using duplicate swabs for microbial enumeration demonstrates the inherent variability between point samples.

TABLE 8 - SURFACE MICROBIAL AND ATP LEVELS ON THE MEAT FILLER MACHINE AND CONVEYOR BEFORE AND AFTER CLEANING AND DISINFECTION BY CONTRACT CLEANERS

Sample Site	Pre-disinfection		Post disinfection	
	TVC (cfu/cm^2)	ATP (rlu/swab)	TVC (cfu/cm^2)	ATP (rlu/swab)
Outside filling machine	$5.6x10^4$	1050	$4.4x10^1$	69
Inside filling machine	$2.4x10^3$	-	$3.6x10^0$	-
Conveyor	$1.4x10^4$	1137	$7.0x10^2$	39

TABLE 9 - SURFACE MICROBIAL AND ATP LEVELS IN THE BEEF PACKING AREA BEFORE AND AFTER DETERGENT FOAM APPLICATION

Sample Site	Pre-disinfection		Post disinfection	
	TVC (cfu/cm^2)	ATP (rlu/swab)	TVC (cfu/cm^2)	ATP (rlu/swab)
Marble chopping board	$8.4x10^3$	-	-	128
Plastic conveyor	$5.3x10^2$	-	$1.9x10^3$	482
Stainless steel conveyor	-	-	$8.2x10^2$	8

(-) Samples absent

TABLE 10 - SURFACE MICROBIAL AND ATP LEVELS IN THE MEAT PREPARATION AREA AFTER CLEANING WITH HIGH PRESSURE WATER (65 - 70°C)

Sample Site	TVC (cfu/cm²)		ATP/Swab
	Swab Site 1	Swab Site 2	rlu
Chopping board (marble)	$1.2x10^5$	$6.8x10^4$	4236
Plastic conveyor 1	$1.9x10^3$	$1.2x10^4$	5191
Stainless steel beside plastic conveyor 1	$6.1x10^6$	$5.1x10^2$	873
Plastic conveyor 2	$1.3x10^4$	-	-
Stainless steel beside plastic conveyor 2	$5.7x10^4$	-	-

(-) Sample absent

FACTORY 4

This site produced a variety of poultry products including cooked chicken pieces and recipe dishes. Table 11 shows the surface microbial levels before cleaning in the high risk area, where cooked chickens were sliced. The figures are generally low. Table 12 shows the levels of microorganisms present on the surfaces after a mid shift clean. The bacteria isolated from this environment are listed in Table 13.

Stainless steel coupons were attached to various locations and left for 6 hours. Table 14 shows the DEM observations from these coupons. Biofilm was detected on the wall in the rack washing area. The gross debris was removed from the racks using high pressure hot water sprays thereby creating aerosols and high levels of humidity. The walls of this area were visibly contaminated with a layer of matter which was in fact a biofilm of microbial cells and product debris. In addition, extensive microcolonies were found on the coupons attached to the bowl chopper guard, the guide rail and the ceiling.

TABLE 11

MICROBIAL LEVELS ON THE SURFACES IN THE HIGH RISK AREA BEFORE CLEANING

Swab Site	Total Viable Count (cfu/cm^2)
Conveyor	$4.6x10^2$
SS side of conveyor	$3.0x10^2$
Conveyor	$3.6x10^0$
Preparation table	$<0.2x10^0$
Preparation table	$<0.2x10^0$
Guide rail packing line	$5.4x10^0$
Wall	$<0.2x10^0$
SS guide rail	$2.5x10^1$
SS guide rail	$1.8x10^1$
Preparation table	$3.2x10^1$
Plastic tray	$1.2x10^2$

TABLE 12 - MICROBIAL LEVELS ON THE SURFACES IN THE HIGH RISK AREA AFTER A MID-SHIFT CLEAN

Swab Site	Total Viable Count (cfu/cm^2)
Conveyor	$<0.2x10^0$
SS conveyor	$5.4x10^5$
Cutting machine	$0.4x10^0$
Preparation table	$<0.2x10^0$
Underneath table	$<0.2x10^0$
Underneath conveyor	$3.0x10^3$
SS guide rail	$2.7x10^4$
Cutting machine crossbar	$>10^4$
SS guide rail	$7.2x10^3$
Preparation table	$9.0x10^1$
SS guide rail	-
Preparation table	$1.1x10^0$
Preparation table	$0.7x10^0$
Stainless steel surface	$0.7x10^0$
Balance	$3.1x10^0$
Preparation table	$4.8x10^0$
SS guide rail	$0.2x10^0$
SS guide rail	$4.0x10^0$
SS surface	$0.4x10^0$
Conveyor belt	$1.3x10^3$

(-) Sample absent

TABLE 13 - IDENTIFICATION OF ISOLATES

Area	Microorganism
Stainless steel conveyor Plastic tray Preparation table	*Hafnia alvei* *Serratia liquefaciens* *Staphylococcus sciuri* *Serratia marcescens*

TABLE 14 - BIOFILM DEVELOPMENT ON SURFACES

Coupon Position (6 hr)	Biofilm Present	Micro-colonies present	Occasional cells present	Product debris present
Side of conveyor	x	x	✓	✓
Side of conveyor	x	x	✓	✓
Preparation table	x	x	✓	✓
Bowl chopper guard	x	✓	✓	✓
Preparation table	x	x	x	✓
Preparation table	x	x	✓	✓
Guide rail	x	✓	x	x
Underneath conveyor	x	x	✓	✓
Weighing table	x	x	✓	x
Ceiling	x	x	x	✓
Ceiling	x	✓	x	x
Wall in rack washing area	✓	✓	x	✓

FACTORY 5

This factory produced a variety of products including meat products, pastry products and pizzas. Stainless steel plates were attached to various locations and left for 6 hours. Table 15 shows the levels of microorganisms detected by DEM. Although biofilms were not detected on any of the coupons, the coupon attached to the egg glaze machine showed extensive microcolony development with a DEM count of 10^6 cells/cm^2. Organisms identified from the factory are shown in Table 16. Table 17 shows the surface microbial and ATP levels before and after cleaning and disinfection. Generally the total viable counts were low (0-10^2 cells/swab); however, the on/off switch of the slicer machine had higher levels of contamination and reflected the poor hygienic design of the switch which makes it practically impossible to clean.

Figure 9 shows the change in the distribution of ATP levels with cleaning. Before cleaning, 20% of the surfaces sampled gave ATP readings in excess of 500rlul however, after cleaning and disinfection only 10% of the surfaces samples gave ATP readings greater than 500rlu. In addition, cleaning increased the percentage of surfaces with 0-50 rlu from 35 to 70%.

TABLE 15 - BIOFILM DEVELOPMENT ON SURFACES

Coupon Position (6hr)	Biofilm present	Micro-colonies present	Occasional cells present	Product debris	% area covered	Count/cm² by DEM
Air conditioner on ceiling in enclosure room	x	x	x	✓	0.1	$6.6x10^4$
Wall of spray room in cooling bay	x	x	x	✓	0.3	$1.9x10^5$
Drain	x	x	x	✓	0.1	$1.6x10^4$
Slicer guide plate	x	x	x	✓	0.8	$5.6x10^5$
Egg glaze machine	x	✓	✓	✓	2.6	$1.8x10^6$
Egg glaze machine	x	x	✓	✓	-	-
Drip tray underneath jelly injecting machine	x	x	x	✓	0.8	$5.3x10^5$

TABLE 16 - IDENTIFICATION OF ISOLATES

Area	Organisms
Air conditioner on ceiling in enclosure room	*Staphylococcus saprophyticus* *Staphylococcus warneri*
Slice guide plate	*Staphylcoccus warneri*
Egg glaze machine	*Bacillus* species
Drip tray underneath jelly injecting machine	*Pseudomonas vesicularis* *Staphylcoccus warneri*

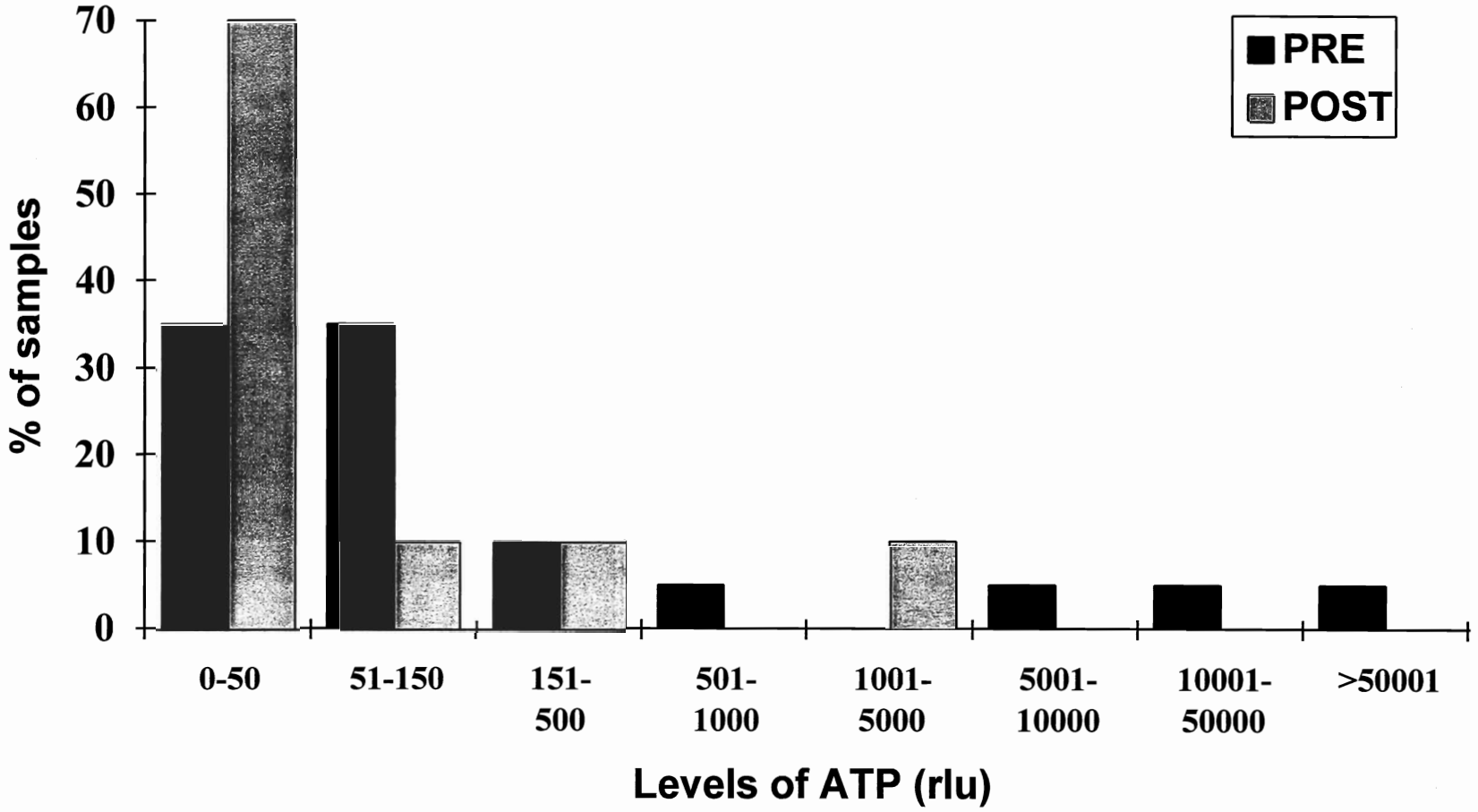
TABLE 17

SURFACE MICROBIAL AND ATP LEVELS BEFORE AND AFTER CLEANING AND DISINFECTION

Sample Sites	Pre Cleaning		Post Cleaning	
	TVC (Cfu/cm²)	ATP (rlu/swab)	TVC (cfu/cm²)	ATP (rlu/swab)
Slicer machine bed	-	-	ND	11
Transfer conveyer	-	-	$0.2x10^0$	17
On/off switch	-	-	$6.0x10^3$	1336
Slicer transfer conveyor	$5.4x10^0$	99	-	5
Slicer machine bed	-	-	$<0.2x10^0$	10
Slicer transfer conveyor	$<0.2x10^0$	123	$<0.2x10^0$	18
Slicer transfer conveyor	$0.2x10^0$	10	$0.2x10^0$	9
Floor near slicer	$6.2x10^2$	10	$1.8x10^2$	96
Stainless steel body of slicer	-	-	$0.7x10^0$	44
Cleaning brush	-	-	$3.9x10^2$	169
Ramp between high and low risk	$2.2x10^2$	528	-	-
Slicer transfer conveyor	$0.2x10^0$	106	-	-
Start button	$6.5x10^3$	314	-	-
Rest for logs before transfer to slicer	$1.5x10^0$	101	-	-
Air stock	$<0.2x10^0$	7	-	-
Washing unit (abattoir)	$5.6x10^5$	340	-	-
Floor (abattoir)	$2.1x10^4$	129774	-	-
Conveyor belt (pastry)	-	9	-	-
Egg glaze machine	$6.8x10^6$	9009	-	-
Margarine table (pastry)	$8.4x10^0$	55	-	-
Balance	$1.0x10^1$	8	-	-
Machine bed	$1.3x10^0$	18	-	-
Start button	$7.6x10^4$	83	-	-
Transfer belt	$1.2x10^1$	93	-	-
Chopping board	$4.1x10^1$	30469	-	-
Floor weigher	$<0.2x10^0$	32	-	-
Swab blank	-	18	-	-

(-) Sample absent

Figure 5 Frequency histogram showing levels of ATP present on the surfaces pre and post cleaning and disinfection at Factory 5



FACTORY 6

This factory produced a variety of products including pizzas and speciality deep fried products. Table 18 shows the surface microbial levels during production. Generally these counts were low, with the exceptions being the overhead conveyor and pizza finishing table which had 10^4 cfu/cm² present. The levels on the conveyor probably reflect the difficulty in gaining access to this piece of equipment for cleaning. Stainless steel plates were attached to the places listed in Table 19. Significant levels of microorganisms were detected on the side of the conveyor and the side of a mixer. Although after 5 hours only occasional microcolonies were visible, potentially at the end of the production run this could have developed significantly. Table 20 lists the organisms identified from the surfaces.

TABLE 18 - SURFACE MICROBIAL LEVELS

Swab Site	Total Viable Count (cfu/cm²)
Pizza finishing table	$2.9x10^4$
Preparation table	$6.0x10^0$
Sauce conveyor	$4.8x10^3$
Conveyor	$5.8x10^2$
Preparation table	$5.9x10^2$
Wall	$2.6x10^2$
Overhead conveyor	$4.5x10^4$
Infeed	$1.8x10^3$
Labelling table	$6.0x10^0$
Plastic tray (clean)	$<0.2x10^0$
Plastic tray (visible moisture)	$5.2x10^2$
Packing table	$<0.2x10^0$
Discharge chute	$<0.2x10^0$
Packing table	$2.8x10^0$
Plastic tray	$4.0x10^0$
Plastic tray	$2.0x10^0$
Packing table	$8.8x10^0$
Tray wash exit	$2.6x10^3$
Cleaned tray	$6.2x10^2$

TABLE 19 - BIOFILM DEVELOPMENT ON SURFACES

Coupon Position (5 hour)	Biofilm present	Micro-colonies present	Occasional cells	Product debris
Side of conveyor	x	✓	✓	x
Side of mixer	x	✓	✓	x
Recirculating batter unit	x	x	✓	x
Side of conveyor	x	x	x	✓
Drain	x	x	x	✓
Onion bhaji forming machine	x	x	x	✓

TABLE 20 - IDENTIFICATION OF ISOLATES

Area	Microorganisms
Conveyor surfaces and preparation tables	*Enterobacter agglomerans* (2 strains) *Acinetobacter calcoaceticus* (3 strains) *Pseudomonas paucimobilis* *Serratia liquefaciens*

FACTORY 7

Factory 7 produced mainly pizzas. The effectiveness of the cleaning protocol (detergent clean followed by disinfection) was assessed by TVC and ATP analysis (Table 21). The results show that in the case of the meat slicer blade and back plate, the sanitation regime reduced the level of microorganisms to below detectable levels and the ATP levels close to background levels. In contrast, cleaning of the chain in the transfer belt was relatively ineffective. Generally for the disinfection stage to be effective, the cleaning stage must be efficient.

TABLE 21 - SURFACE MICROBIAL AND ATP LEVELS BEFORE AND AFTER CLEANING AND DISINFECTION

Sample Site	Before Cleaning		After Cleaning		After Disinfection	
	TVC (Count/cm²)	ATP (rlu/swab)	TVC (Count/cm²)	ATP (rlu/swab)	TVC (Count/cm²)	ATP (rlu/swab)
Preparation table	$6.8x10^4$	298	-	-	$1.2x10^2$	34
Meat slicer blade	$8.8x10^2$	46	-	-	$<0.2x10^0$	5
Meat slicer back plate	$6.0x10^4$	47	-	-	$<0.2x10^0$	14
Transfer line edge	$3.0x10^2$	111	$2.8x10^2$	9	$<0.2x10^0$	14
Beneath transfer line	$2.4x10^2$	61	$5.6x10^2$	20	$9.2x10^1$	5
Chain on transfer line	$1.0x10^3$	154	$1.0x10^3$	31	$6.8x10^1$	16
Plastic conveyor belt	$2.2x10^2$	104	$2.8x10^0$	4	$4.4x10^1$	43
SS edge of conveyor belt	$1.1x10^3$	266	$1.4x10^4$	372	$4.0x10^0$	17

(-) Sample absent

FACTORY 8

Two production units were studied at Factory 8, the mashed potato area and the fish cake area. Table 22 shows the efficiency of the cleaning regime in both these areas. The mashed potato production area by its nature was a very humid environment. Assessment of the cleaning protocol shows that a reduction of less than two log orders was achieved and the ATP levels also remained high after cleaning. Stainless steel coupons were attached to various locations in this area (Table 24). Biofilms were detected on the fish cake conveyor and the ceiling near the air vent. Figure 6 shows the position of the stainless steel coupon near the air vent in the ceiling of the potato washing area. Figure 7 shows the extensive biofilm that developed on the stainless steel surface within 24hrs. Condensation was visible on the stainless steel surfaces and the ceiling and was probably the major cause of the extensive surface development. This example illustrates that extensive biofilms can develop relatively rapidly on environmental surfaces, including ceilings, in food processing environments. The biofilm consisted of microcolonies of yeasts and bacteria (Table 23). Figure 8 shows the fish cake conveyor and the position of the stainless steel coupon. Figure 9 shows the level of surface contamination after the relatively short exposure time of 1.5hr and shows that bacterial contamination can build up very rapidly.

Monitoring of the cleaning regime in the fish cake production area showed only a small reduction in microbial numbers after cleaning, although disinfection reduced these levels to below detectable levels (Table 22).

TABLE 22 - SURFACE MICROBIAL LEVELS BEFORE AND AFTER CLEANING AND DISINFECTION

Sample Site	Before Cleaning		After Cleaning		After Disinfection	
	TVC (cfu/cm²)	ATP (rlu/ swab)	TVC (cfu/cm²)	ATP (rlu/ swab)	TVC (cfu/cm²)	ATP (rlu/ swab)
Masher conveyor	$1.3x10^2$	10983	-	-	$1.8x10^2$	3432
Mixer conveyor	$1.0x10^2$	374	-	-	$2.0x10^0$	207
Ceiling	$6.4x10^5$	-	-	-	-	-
Batter coated	$6.4x10^3$	30	$1.7x10^3$	7	$<0.2x10^0$	2
Cod cake conveyor	$1.5x10^1$	139	$5.2x10^3$	98	$<0.2x10^0$	4

TABLE 23 - IDENTIFICATION OF BIOFILM ORGANISMS

Area	Microorganism
Ceiling in potato mashing room	*Flavobacterium* species *Pseudomonas fluorescens* Yeast
Cod cake, press out onto conveyor	*Providencia rettgeri*

TABLE 24 - BIOFILM DEVELOPMENT ON SURFACES

Coupon Position	Time (hours)	DEM Count cells/cm²	% Coverage	Biofilm
Tuna cake, press out onto conveyor	1.5	$1.4x10^4$	0.0	x
Tuna cake, batter coating	3.75	$6.7x10^4$	0.1	x
Cod cake, press out onto conveyor	1.5	$4.6x10^6$	11.3	✓
Cod cake, batter coating	1.0	$4.6x10^4$	0.1	x
From masher to conveyor	6.5	$6.7x10^4$	0.1	x
From conveyor to mixer	6.5	$1.5x10^4$	0.0	x
Ceiling near vent	24	$5.5x10^5$	3.6	✓
Drain on floor	24	$5.2x10^5$	0.8	x

Figure 6 Air extraction vent in the ceiling of the potato mashing area of factory 8 showing the position of the stainless steel coupons

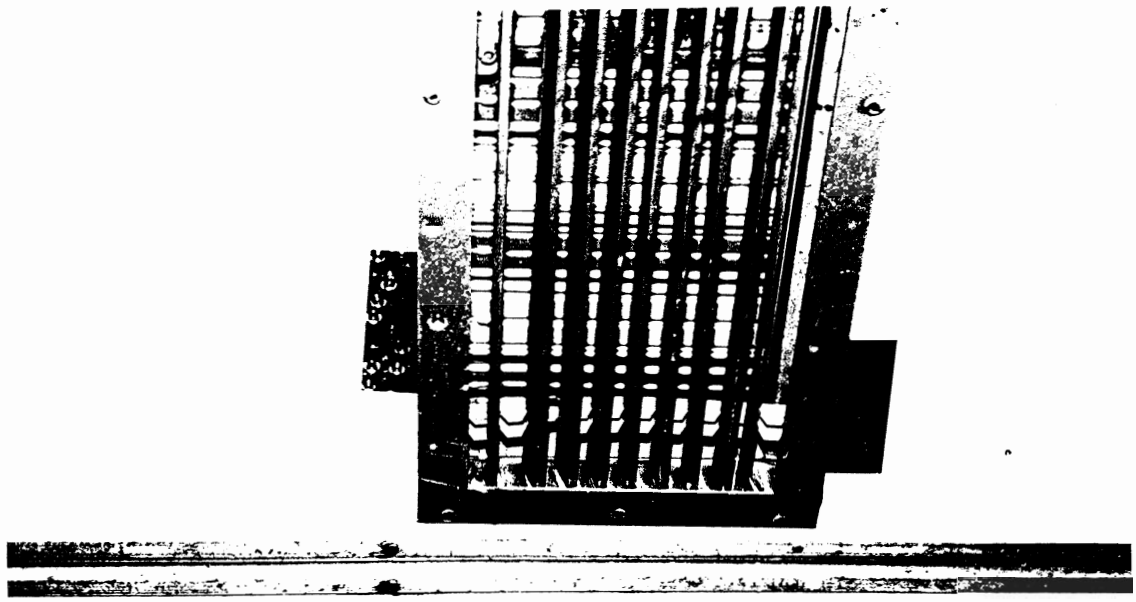


Figure 7 Acridine orange stained coupon removed from the ceiling of factory 8 after 24 hours

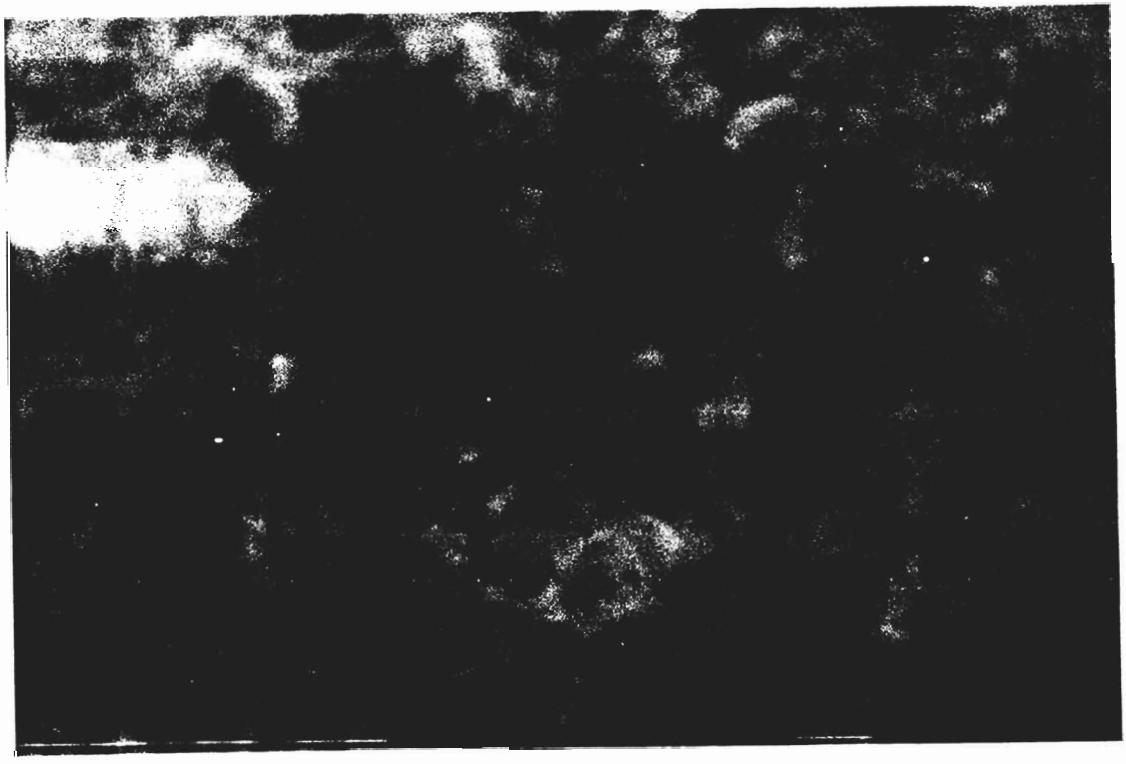


Figure 8 Fish cake conveyor of factory 8 showing the position of the stainless steel coupons

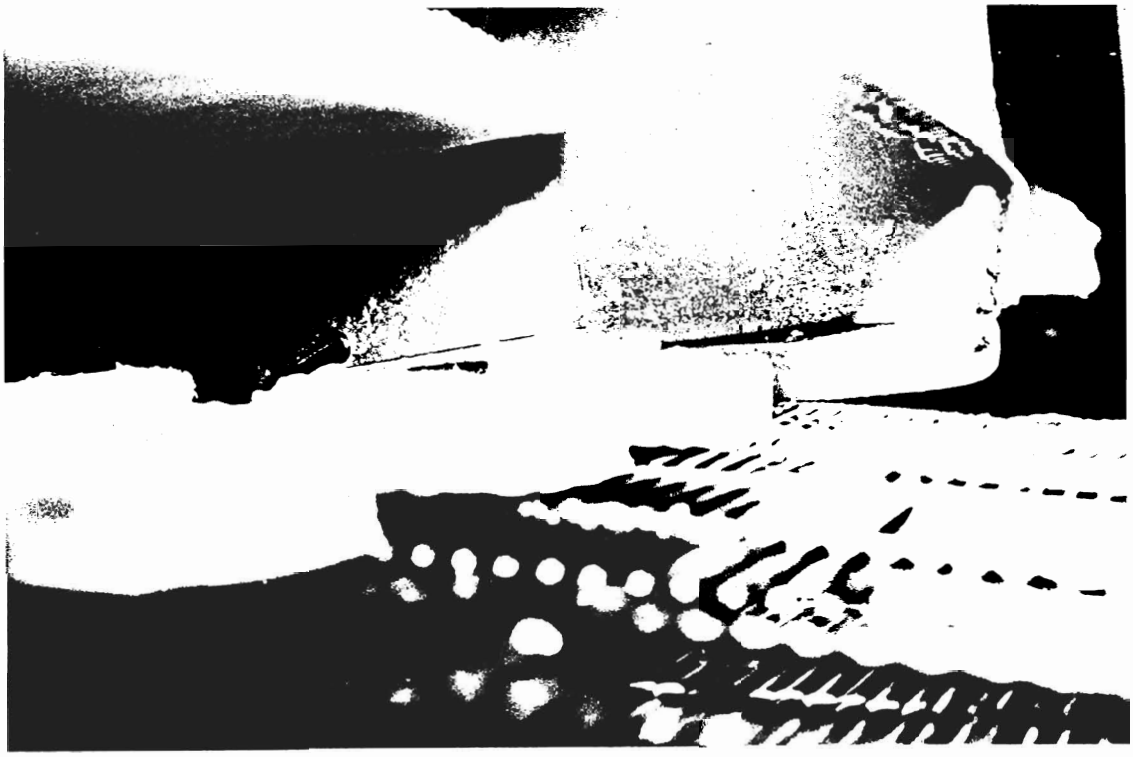


Figure 9 Acridine orange stained coupon removed from the side of the fishcake conveyor after 1.5hr

FACTORY 9

This site produced a variety of fish products including frozen fish fillets, formed fish portions and fish cakes. Table 25 shows the surface microbial levels before and after cleaning and disinfection. Although the levels of microorganisms on the surfaces before cleaning are only of the order of 10^2 cfu/cm^2 cells/swabs, the cleaning regime reduced this level by up to 2 log orders. A small increase in the TVC of the stainless steel conveyor was detected after cleaning but this is probably due to the problems associated with swabbing adjacent sites. Stainless steel plates were attached to various locations for 8 hours; however, the only coupon that showed significant levels of microorganisms was the stainless steel plate left in the drain (Table 26). Table 27 lists the organisms identified from this environment.

TABLE 25

SURFACE MICROBIAL LEVELS BEFORE AND AFTER CLEANING AND DISINFECTION

Sample Site	TVC (cfu/cm²)	
	Pre-cleaning	Post-cleaning
Fish cake kitchen gardner feed belt	$2.0x10^2$	$0.2x10^0$
SS conveyor belt	$3.4x10^2$	-
Edge of forming plate	$6.5x10^2$	$0.2x10^0$
Wall	$<0.2x10^0$	-
Surface neat fryer in feed	$4.3x10^1$	-
Fish cake former hood	$2.3x10^2$	-
SS conveyor belt	$5.0x10^1$	-
SS guide rail	$0.2x10^0$	$<0.2x10^0$
Inside batter enrober	-	$0.2x10^0$
Fish cake gardner belt	$1.7x10^2$	-
Scraper for galley tub	$4.7x10^1$	-
Guide on top of galley	$7.7x10^1$	-
SS conveyor	$1.7x10^2$	-
SS conveyor	$4.6x10^1$	-
Preportioner blade guide	$8.6x10^1$	-
Guide rail	$2.9x10^2$	$3.1x10^1$
SS conveyor	$0.5x10^0$	$2.8x10^0$
Plastic guide rail	$0.2x10^0$	$0.2x10^0$
Batter enrober	-	$0.2x10^0$
Wall	-	$4.7x10^0$
Near batter enrober blower	$0.5x10^0$	$7.5x10^0$
Guide rail	$5.1x10^1$	-
Conveyor belt	$3.6x10^2$	-
Slicing machine	$<0.2x10^0$	-

(-) Sample absent

TABLE 26 - BIOFILM DEVELOPMENT ON SURFACES

Coupon Position (8hr)	Epifluorescent Microscope Observations			
	Biofilm present	Micro-colonies present	Occasional cells present	Product debris
Fish cake kitchen, gardner feel belt	x	x	✓	✓
Fish cake kitchen, gardner feed belt	x	x	✓	✓
Edge of forming plate	x	x	✓	✓
Batter enrober	x	x	✓	✓
Batter enrober	x	x	x	✓
Frier infeed	x	x	x	✓
Preportioner blade guide	x	x	✓	✓
Underneath batter enrober	x	x	✓	✓
Drain	x	✓	✓	✓
Preportioner blade guide	x	x	x	✓
Batter enrober	x	x	x	✓
Driptank	x	x	x	✓
Batter enrober	x	x	x	✓

TABLE 27 - IDENTIFICATION OF ISOLATES

Area	Microorganism
Conveyor side	*Citrobacter freundii*
Edge of forming plate	*Enterobacter agglomerans*
Batter enrober	*Staphylococcus auricularis*
SS guide rail	*Pseudomonas fluorescens*
Fryer in feed	*Proteus vulgaris*
Edge of forming plate	*Kluyvera* species
SS conveyor belt	*Pseudomonas paucimobilis*

FACTORY 10

Factory 10 processed a protein substitute. The site comprised a low risk (normal) area where the protein substitute was mixed with various ingredients and a low temperature high care area where the product was packaged. In the low risk (normal) area the external surfaces of the mixer were exposed to moisture and product splashes during operation. A high level of microorganisms was found on the ledge surrounding this machine (Table 28).

The counts in the high care area, where the temperature was approximately 4°C, were generally 10^1-10^3 cfu/cm^2. However, higher numbers were found on the underside of the conveyor and wall bumper. The under surface of the plastic conveyor was of a fibrous nature, presenting crevices for the retention of microorganisms. Table 29 shows the ineffectiveness of the liquid detergent cleaning regime used on the bucket conveyor. This regime only produced a one log reduction in surface numbers.

Biofilm development was assessed at the sites described in Table 30. The only area where biofilms were detected was on the undersurface of the ledge surrounding the mixer after 20 hours, by which time the area coverage was in excess of 50%.

Microorganisms identified in the biofilm are shown in Table 31.

TABLE 28

SURFACE MICROBIAL LEVELS IN VARIOUS AREAS OF FACTORY 10

Sample Site	Total Viable Count (cfu/cm^2)
Normal Care Area	
Mixer - upper surface of ledge	$7.8x10^5$
Mixer - beneath ledge	$2.7x10^7$
Low temperature mixer	$1.1x10^3$
Forming machine	$2.8x10^2$
High Care Area	
Plastic belt surface	$8.4x10^1$
Plastic belt surface	$1.2x10^2$
Underside of belt surfaces	$3.8x10^6$
Inner stainless steel surface near belt	$1.3x10^2$
Cross bar under belt	$7.0x10^1$
Slipway before conveyor	$6.3x10^1$
Top surface of wall bumper	$3.2x10^3$
Under surface of wall bumper	$2.6x10^6$

TABLE 29 - MONITORING CLEANING EFFICIENCY OF THE BUCKET CONVEYOR BY SWABBING

Sample Site	TVC cfu/cm²	
	Before Cleaning	After Cleaning
Underside of belt (fibrous material)	1.2×10^6	3.5×10^5
Inner stainless steel surface adjacent to belt	1.2×10^4	1.0×10^3

TABLE 30 - BIOFILM DEVELOPMENT ON STAINLESS STEEL COUPONS

Coupon Position	Exposure Time (hours)	DEM Count cells/cm²	% Coverage	Biofilm Development
Mixer - upper surface of ledge	3	5.7×10^5	0.5	x
	20	5.0×10^6	4.8	x
Mixer - under surface of ledge	3	1.1×10^6	1.0	x
	20	$>5.9 \times 10^7$	56.1	✓
Low temperature mixer	20	5.5×10^5	0.3	x
Bucket conveyor - cross bar below belt	20	4.9×10^5	0.4	x

TABLE 31 - IDENTIFICATION OF BIOFILM ORGANISMS

Sample Site	Microorganisms
Mixer - under surface of ledge	*Pseudomonas fluorescens* *Xanthomonas maltophilia (Pseudomonas maltophilia)* *Staphylococcus warneri*

FACTORY 11

This further processing site sliced and packaged cheese. Table 32 shows the surface microbial and ATP levels before and after cleaning and disinfection. The effectiveness of the cleaning regime appears to be variable with log reductions between 0 and 3 log orders. The underneath of the conveyor belt had particularly high levels of microorganisms (in excess of 10^6 cells/cm²) and even after cleaning and disinfection 10^3 cells/cm² remained, probably due to the difficulties in cleaning these belts. The organisms identified from this environment are shown in Table 33.

TABLE 32

SURFACE MICROBIAL AND ATP LEVELS BEFORE AND AFTER CLEANING AND DISINFECTION

Sample Site	Pre Cleaning			Post Cleaning		
	TVC (cfu/cm²)	Coliform (cfu/cm²)	ATP (rlu)	TVC (cfu/cm²)	Coliform (cfu/cm²)	ATP (rlu)
Painted hatch	$1.2x10^2$	$<0.2x10^0$	37	$0.6x10^0$	$<0.2x10^0$	5
SS plastic rollers	-	$<0.2x10^0$	-	$0.2x10^0$	$<0.2x10^0$	157
Plastic rollers	$1.0x10^3$	$<0.2x10^0$	-	$0.4x10^0$	$<0.2x10^0$	12
Conveyors belt	-	-	735	$8.4x10^2$	$0.2x10^0$	8
SS guide rails	$4.0x10^2$	$<0.2x10^0$	-	$1.3x10^3$	$2.1x10^1$	15
Spacers	$1.2x10^3$	$<0.2x10^0$	13	$2.4x10^1$	$<0.2x10^0$	15
Conveyor (upper)	$4.0x10^2$	$0.8x10^0$	841	$3.2x10^2$	$1.2x10^1$	6
Conveyor (underneath)	$3.8x10^4$	-	-	-	-	-
Cutting wires	$3.5x10^2$	$<0.2x10^0$	-	$4.0x10^1$	$0.6x10^1$	-
SS side (joint)	$5.2x10^3$	$0.4x10^0$	-	$9.6x10^1$	$<0.2x10^0$	-
SS side	$1.0x10^3$	$<0.2x10^0$	-	$8.0x10^1$	$4.2x10^1$	-
Plastic receiver	$4.0x10^2$	$<0.2x10^0$	-	$8.4x10^2$	$1.2x10^1$	-
Sink	$5.6x10^2$	$3.6x10^1$	301	-	-	38
Conveyor (underneath)	$1.8x10^6$	-	78073	$2.2x10^3$	-	46102
Waste tray	$1.6x10^3$	$2.6x10^0$	-	$2.2x10^2$	$1.2x10^0$	-
Cutter	$1.4x10^2$	$5.4x10^0$	682	$2.3x10^2$	$5.2x10^0$	9

(-) Sample absent

TABLE 33 - IDENTIFICATION OF ISOLATES

Area	Microorganisms
Spacers	*Bacillus* sp.
Underneath conveyor	*Aeromonas hydrophila*
Conveyor (upper)	*Staphylococcus warneri*
Cutting wires	*Pseudomonas pickettii*
Plastic receiver	*Pseudomonas aeruginosa*

FACTORY 12

This factory produced a variety of salad products. The levels of microorganisms detected on the surfaces during production are shown in Table 34. Particularly high levels were detected on the stainless steel surface of the potato dicing machine. This machine was in a room with the steamers and due to inefficient extraction systems, condensation was present, and the potato dicing machine itself splashed water and product onto surrounding surfaces. High levels were also found on the stainless steel guide on the cabbage line and on the floor of the spray cooler. Racks carrying trays of diced potato were pushed into this cooler, and therefore contamination was carried on the rollers of the racks. The potato in the trays, particularly near the bottom of the racks, could become contaminated through splashes from the floor. Stainless steel coupons were attached to a variety of locations for either 6 or 10 hours, but the only site that showed significant microbial development was the coupon placed in the drain (Table 35). Although the TVC result for the stainless steel guide on the cabbage line was relatively high, cells were not visible on the surface by DEM examination. The cells may be associated with product debris and be indistinguishable under the microscope. The organisms identified from this site are shown in Table 36.

TABLE 34 - SURFACE MICROBIAL LEVELS

Sample Site	Total Viable Count (cfu/cm²)
Plastic chopping board	$2.0x10^3$
Underneath slicer	$9.0x10^0$
Underneath slicer	$3.1x10^1$
SS guide on cabbage line	$6.8x10^4$
Conveyor belt	$4.8x10^2$
Wall above steamers	$<0.2x10^0$
Potato dicing machine	$1.0x10^5$
Edge of pallet	$3.2x10^2$
Edge of pallet	$1.0x10^0$
Cabbage hopper	$1.9x10^3$
Wall in spray cooler	$2.4x10^3$
Floor in spray cooler	$2.4x10^6$
Wall	$8.2x10^0$

TABLE 35 - BIOFILM DEVELOPMENT ON SURFACES

	Epifluorescent Microscope Observations			
Coupon Position (coupons 1-8 10hr) (coupons 9-20 6hr)	Biofilm present	Microcolonies present	Occasional cells present	Product debris present
SS guide cabbage line	x	x	x	✓
Wall above steamers	x	x	✓	✓
Drain	x	✓	✓	✓
Underneath slicer	x	x	x	✓
Drain	x	x	✓	✓
Underneath slicer	x	x	x	✓
Potato dicing machine	x	x	✓	x
Inside spray cooler	x	x	x	x
Table leg	x	x	x	✓
SS side conveyor	x	x	✓	✓
Grill by air outflow	x	x	✓	x
Grill for air outflow	x	x	✓	x
Ceiling	x	x	✓	x
Air sock chiller unit	x	x	✓	x
Wall of spray chiller	x	x	✓	✓
Barrier between cook/chill	x	x	✓	✓
Splash guard	x	x	x	x
Cross bar under cooker	x	x	✓	✓
Blast chiller	x	x	x	x
End of conveyor	x	x	x	✓

TABLE 36 - IDENTIFICATION OF ISOLATES

Area	Microorganisms
Vegetable preparation table	*Agrobacterium tumefaciens* *Hafnia alvei*
Potato dicing machine	*Flavobacterium indologines*
Floor in spray cooler	*Aeromonas hydrophila*

FACTORY 13

This factory produced a variety of salad products. Tables 37 and 38 show the surface microbial levels pre and post cleaning. Before cleaning, the chopping board, the surface above the waste conveyor, the exit chute from the slicer, the plastic conveyor and a plastic container showed levels of microorganisms in excess of 10^5 cfu/cm². The preparation of these salad products is labour intensive, requiring an operative to place a particular component of the salad mix in the tray as it passes along a conveyor. The swab result from the gloves of one such person gave a count in excess of 10^4 cfu/cm². After cleaning the levels of organisms detected ranged from 0-10^3 cfu/cm². Table 39 lists the organisms identified from this factory.

Stainless steel coupons were attached to a variety of locations and the DEM results are shown in Table 40. Although biofilms were not detected, micro-colony development was observed on coupons attached to the preparation table, the washer outlet, the side of the conveyor (after 10 hours) and the side of the horizontal conveyor (after 10 hours).

TABLE 37 - SURFACE MICROBIAL LEVELS PRE CLEANING

Swab Site	TVC (cfu/cm²)
Above waste conveyor	$9.4x10^5$
Chopping board	$4.2x10^6$
Preparation table	$8.2x10^4$
Wall	$2.2x10^1$
Exit chute to vegetable slicer	$8.8x10^5$
Outlet of washer	$1.8x10^0$
Side of washer	$<0.2x10^0$
Shaker washer	$<0.2x10^0$
Top conveyor	$3.7x10^3$
Plastic bucket on multipack	$1.2x10^2$
Plastic guide rails	$1.3x10^3$
SS guide	$4.2x10^3$
Plastic conveyor	$5.9x10^4$
Plastic conveyor	$2.1x10^6$
Condensate on window ledge	$<0.2x10^0$
Plastic container	$8.1x10^5$
Plastic gloves of handler	$6.0x10^4$

TABLE 38 - SURFACE MICROBIAL LEVELS POST CLEANING

Swab Site	Total Viable Count (cfu/cm^2)
Chopping board	$7.2x10^2$
Preparation table	$6.9x10^1$
Exit chute to vegetable slicer	$9.0x10^2$
Stainless steel bottom of conveyor	$0.7x10^0$
Plastic sides of conveyor	$<0.2x10^0$
Conveyor belt	$1.0x10^1$
SS tray guide	$0.4x10^0$
Weigh heads	$0.4x10^0$
Weigh heads	$4.3x10^0$
Blade on slicer	$0.2x10^0$
Tray guide	$3.6x10^0$
Balance	$0.2x10^0$
Plastic tray	$0.2x10^0$
Window ledge	$2.9x10^0$
Batch washer	$0.2x10^0$
Table	$1.4x10^0$
Plastic mould	$<0.2x10^0$
Table leg	$1.2x10^3$

TABLE 39 - IDENTIFICATION OF ISOLATES

Area	Microorganisms
Stainless steel sides of line	*Klebsiella pneumoniae*
	Kluyvera species
	Enterobacter agglomerans (2 strains)
	Citrobacter freundii
	Pseudomonas fluorescens
	Pseudomonas aeruginosa
Table	*Acinetobacter calcoaceticus*
Plastic conveyor	*Klebsiella pneumoniae*
Preparation table	*Serratia plymuthica*
Side of conveyor	*Pseudomonas fluorescens* (3 strains)
	Enterobacter agglomerans
	Pseudomonas vesicularis

TABLE 40 - BIOFILM DEVELOPMENT ON SURFACES

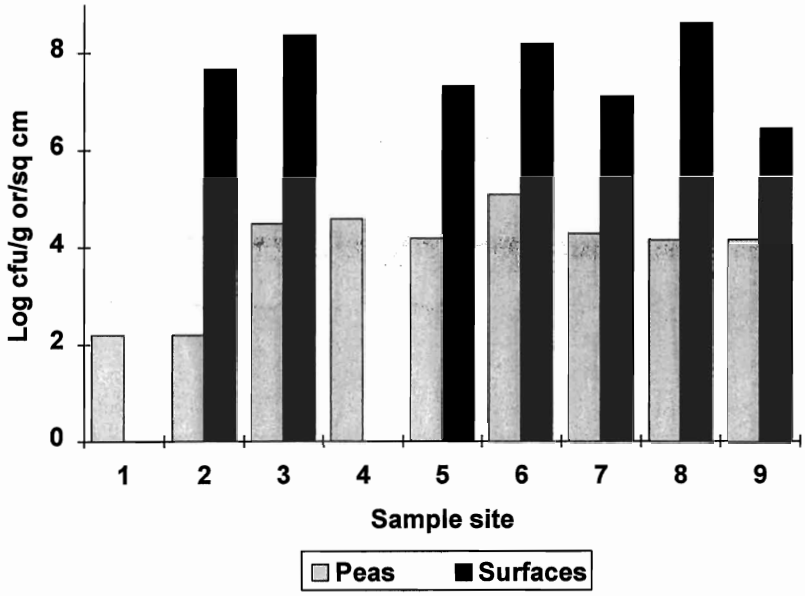
Coupon Position	Time (hr)	Biofilm present	Micro-colonies present	Occasional cells present	Product debris
Preparation table	5	x	✓	✓	✓
Preparation table	5	x	x	✓	✓
Slicer outlet	5	x	x	x	✓
Washer outlet	5	x	✓	✓	✓
Floor	5	x	x	✓	✓
Side of conveyor (top)	5	x	✓	✓	✓
Side of conveyor (horiz)	5	x	x	✓	✓
Side of goose conveyor	5	x	x	x	x
SS guide rail	5	x	x	x	✓
Floor	5	x	x	✓	✓
Side of conveyor	3	x	x	x	✓
Side of conveyor	5	x	x	✓	✓
Side of conveyor	7	x	x	✓	✓
Side of conveyor	10	x	✓	✓	✓
Side of horizontal conveyor	3	x	x	x	✓
Side of horizontal conveyor	5	x	x	✓	✓
Side of horizontal conveyor	7	x	x	✓	✓
Side of horizontal conveyor	10	x	✓	✓	✓

FACTORY 14

This site produced frozen peas. Fresh peas were delivered to the site, and were shaken to remove foreign matter and washed. This part of the operation took place outside the factory. The peas then entered the factory, were blanched and cooled, passed along several conveyors and finally entered the freezers. Figure 10 shows the microbial levels on the surface on the process line and the peas as the peas passed from the blancher to the exit from the freezers. The level of contamination on the surfaces remained consistently high along the process line at around 10^7 - 10^8 cfu/swab. In contrast the level of contamination on the peas was low until the peas left the cooler system and passed onto the conveyor belt system, where the level of contamination increased to 10^4-10^5 cfu/g. In addition to the total viable count, a count was made of the nine most common isolates at each site and Figure 11 shows the incidence of these isolates on the equipment surfaces and the peas. *Klebsiella pneumoniae* appeared most commonly on the surfaces and the peas. Isolate 3, the Gram positive rod, appeared to be associated with the surfaces rather than the peas. In contrast, *Trichosporan beigelli* appeared to be associated with the peas rather than the stainless steel surfaces. These differences may relate to individual species surface phenomena that cause these species to attach to one type of surface preferentially. Similarly, Dunsmore *et al* (1981) found that micrococci dominated cleaned stainless steel surfaces whilst rubber surfaces were dominated by flavobacteria and pseudomonads.

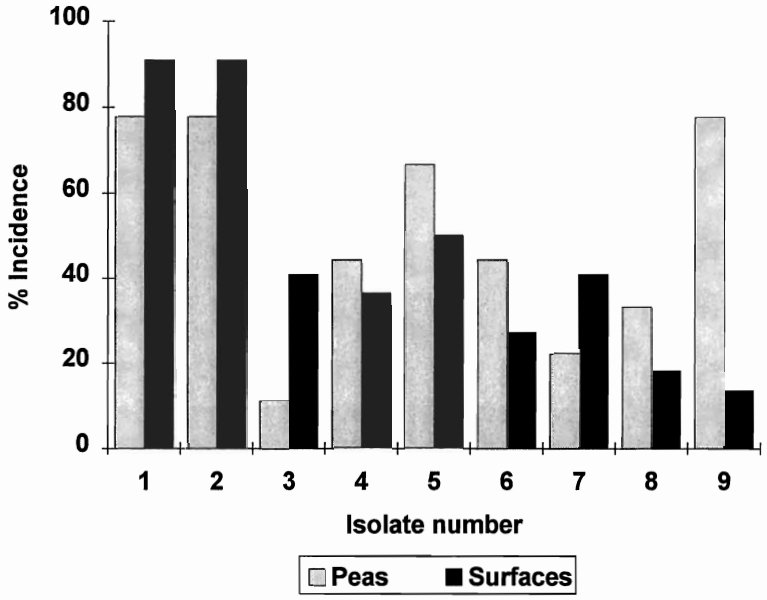
Table 41 shows the DEM observations of coupons attached to various positions. Figure 12 shows the exit from the blancher system and Figure 13 shows the position of the stainless steel coupons on the side of the inspection belt conveyor. The level of contamination present on the surfaces after 48 hours is shown in Figure 14. High levels of microorganisms were observed on almost all the coupons and most of the coupons showed microcolony development. The fact that the level of contamination on the peas exiting the cooler was low, but the level of contamination increased significantly as the peas passed along the conveyor belting system and surfaces which had high levels of contamination demonstrates the link between surface contamination and the product contamination.

Figure 10 Level of contamination on the peas and surfaces along the process line (Surfaces were not sampled at sites 1 and 4)



Sample site No.	Site description
1	Exit to blancher (before water spray)
2	Exit to blancher (after water spray)
3	Cooler outfeed
4	Transfer shaker
5	Inspection belt shaker
6	Mezzanine chute
7	Transfer shaker
8	Transfer shaker to freezer
9	Freezer exit chute

Figure 11 Comparison of the incidence of the nine most common isolates on the surfaces and the peas



Isolate No.	Isolate
1	*Klebsiella pneumoniae* strain 1
2	*Klebsiella pneumoniae* strain 2
3	Gram positive rod
4	*Pseudomonas fluorescens*
5	Gram negative rod
6	Gram negative rod
7	*Vibrio fluvialis*
8	*Enterobacter agglomerans*
9	*Trichosporon beigelii*

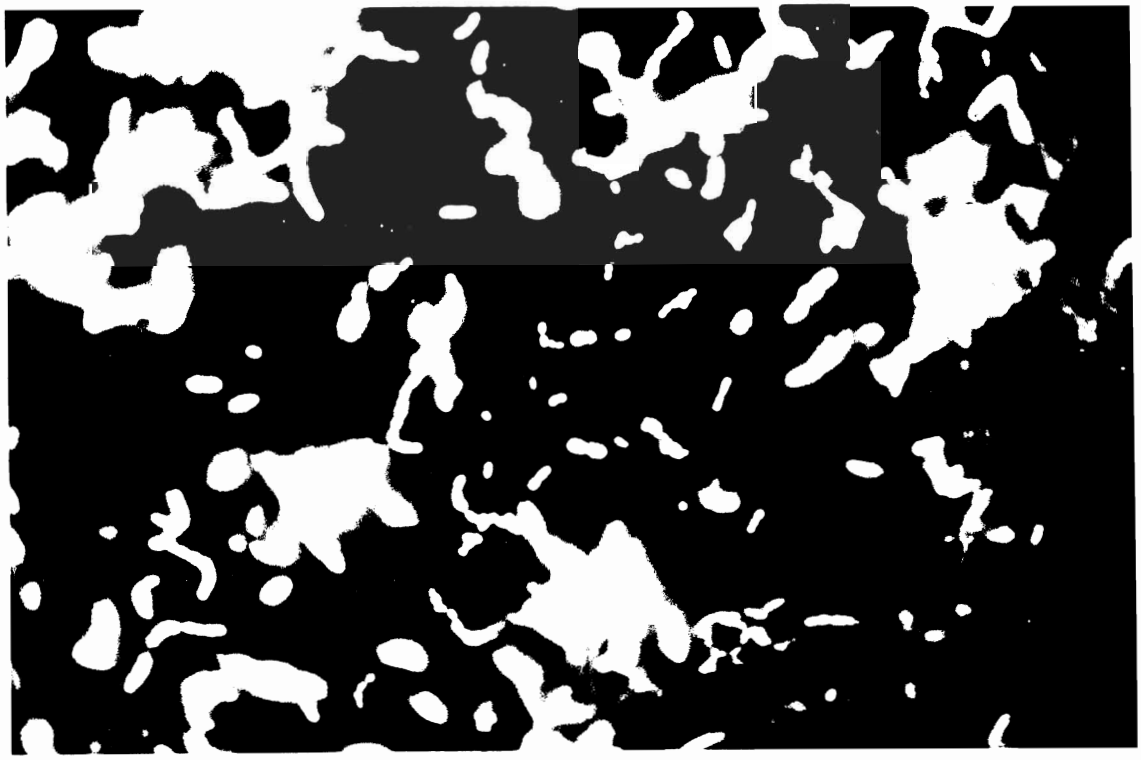
TABLE 41 - BIOFILM DEVELOPMENT ON SURFACES

Coupon Position	Time (hr)	Biofilm present	Micro-colonies present	Occasional cells present	Product debris
Inspection belt guard	11	x	✓	✓	✓
	17	x	✓	x	✓
	20	x	✓	x	✓
	24	x	✓	x	✓
Inspection belt guard	24	x	✓	x	✓
	48	✓	✓	x	✓
Mezzanine chute outlet	24	x	✓	✓	✓
	48	x	✓	x	✓
Inspection belt guard	6	x	x	✓	x
	14	x	✓	✓	x
	49	x	✓	✓	x

Figure 12 Transfer of blanched peas from the blancher to the conveyor belt system in factory 14

Figure 13 Stainless steel coupons attached to the side of the inspection belt conveyor of factory 14.

Figure 14 Acridine orange stained coupon from the side of the inspection belt, removed after 48hr



FACTORY 15

This factory produced a variety of biscuits. The microbial and ATP levels on the surfaces during production are shown in Table 42. During production the total viable counts ranged from 10^2 to 10^6 cells per cm², although higher levels were detected in the steam clean room. The cream adding machine was cleaned using hot water which reduced the levels of microorganisms by nearly three log orders.

The steam clean room was not totally separated from the production area. In this cleaning room, the pipes from the chocolate line were cleaned by high pressure steam, which obviously generated a humid and warm environment. This room was visibly contaminated with surfaces covered in a biofilm of product and bacterial cells. The total viable count of a ledge in this area showed in excess of 10^7 cells/cm². Table 43 shows that after 16 hours a biofilm covering 20% of the coupon was found on the coupon left in the steam clean room. The organisms detected in this biofilm are detailed in Table 44.

TABLE 42 - SURFACE MICROBIAL AND ATP LEVELS

Sample Site		Total Viable Count (cfu/cm²)	ATP (rlu)
Below conveyor - line 1		$3.6x10^3$	34
Discarded biscuit chute		$4.2x10^3$	689
Below conveyor - line 2		$1.5x10^6$	166
Cream adding machine	before cleaning	$8.0x10^3$	329
	after cleaning	$2.3x10^1$	52
Beneath conveyor		$5.3x10^2$	-
Leg adjacent to blender		$6.3x10^4$	-
Platform adjacent to blender		$3.3x10^4$	-
Steam clean room		$7.4x10^7$	-
Drain		$6.2x10^5$	-

(-) Sample absent

TABLE 43 - BIOFILM DEVELOPMENT ON SURFACES

Coupon Position	Time (hr)	DEM Count cells/cm²	% Coverage	Biofilm present
Below conveyor - line 1	15	$3.0x10^6$	2.2	x
Discarded biscuit chute	15	$7.0x10^5$	0.4	x
Below conveyor - line 2	15	$4.2x10^6$	2.8	x
Cream adding machine	15	$8.3x10^5$	0.6	x
Below conveyor - line 3	16	$4.0x10^6$	3.2	x
Leg adjacent to blender	16	$1.9x10^6$	1.6	x
Platform adjacent to blender	16	$1.4x10^5$		x
Cream room	16	$9.3x10^6$	4.2	x
Steam clean room	16	$>1.4x10^7$	20.2	✓
Drain	16	$2.5x10^6$	2.9	x

TABLE 44 - MICROBIAL IDENTIFICATION OF BIOFILM ORGANISMS

Sample Site	Organism
Steam clean room	*Pseudomonas fluorescens* *Flavobacterium indologenes* *Acinetobacter calcoaceticus* *Staphylococcus saprophyticus*

FACTORY 16

This factory produced a type of biscuit, and as higher than usual levels of microorganisms were reported in the finished product, surface samples, air samples, temperature and humidity readings were taken at various locations.

Table 45 shows the surface microbial levels during production for two weeks, and after cleaning for week one. The results show that significant levels of microorganisms developed on the surfaces over the week, although these levels were effectively reduced by the cleaning regime and were not repeated the following week. The air sample data (Table 46) confirmed the particularly high count detected from the air bed. Pieces of stainless steel were cut into the shape of the guide bars at the side of the air bed. These were attached in place of the original guide bars for various intervals, and examined by DEM. Very little was observed on these pieces of stainless steel (Table 47). Table 48 lists the bacteria identified from the surface samples.

The particularly high surface microbial levels detected in week one were attributed to the production of biscuits with a higher moisture content due to the malfunction of processing equipment. These results illustrate that although the processing failure was before the packing area, significant levels of contamination were present on the surfaces throughout the packaging area. In excess of 10^6 cfu/cm^2 were detected on the air bed, guide bar, rivet hole and the divided section, all of which were in direct contact with product. This emphasises the importance of process control and effective cleaning and disinfection regimes.

TABLE 45 - SURFACE MICROBIAL LEVELS DURING PRODUCTION AND AFTER CLEANING AND DISINFECTION

Sample Sites	Total Viable Count (cfu/cm²)					
	Week 1				Week 2	
	Day 1	Day 2	Day 5	After cleaning	Day 1	Day 5
Conveyor entering packing area	$2.8x10^2$	$1.0x10^8$	$1.7x10^8$	-	$3.5x10^1$	$4.0x10^2$
First arc conveyor	$1.3x10^2$	$3.1x10^3$	$2.7x10^2$	$5x10^0$	$3.5x10^1$	$6.8x10^2$
Straight conveyor	$4.5x10^1$	$1.6x10^2$	$1.5x10^1$	$5.0x10^0$	$5.5x10^1$	$1.5x10^1$
Arc conveyor	$1.3x10^3$	$3.5x10^2$	$1.7x10^2$	-	$4.0x10^1$	$1.9x10^2$
Straight conveyor	$2.0x10^1$	$1.5x10^1$	$<0.2x10^0$	$2.5x10^1$	$3.3x10^2$	$5.0x10^0$
Arc conveyor	$2.0x10^1$	$2.5x10^1$	$7.5x10^1$	$1.5x10^1$	$1.6x10^2$	$6.7x10^2$
Straight conveyor	$8.5x10^1$	$3.5x10^1$	$1.6x10^5$	$9.5x10^1$	$<0.2x10^0$	$<0.2x10^0$
Air bed	$7.5x10^1$	$2.5x10^3$	$1.3x10^6$	$1.0x10^1$	$5.5x10^1$	$4.0x10^1$
Guide bar	$2.2x10^2$	$2.9x10^7$	$1.8x10^4$	$<0.2x10^0$	$5.1x10^2$	$1.5x10^1$
Bottom of divided section	$1.6x10^3$	$1.2x10^6$	$3.9x10^5$	$5.0x10^1$	$1.5x10^1$	$6.0x10^1$
Rivet hole	$1.5x10^1$	$4.6x10^8$	$1.3x10^5$	$1.5x10^2$	$6.5x10^1$	$<0.2x10^0$
Reject chute	$6.0x10^1$	$8.0x10^1$	$1.6x10^5$	$2.4x10^2$	$5.5x10^1$	$1.0x10^1$
Edge of divided section	-	-	-	-	$1.5x10^1$	$<0.2x1^0$
Support bar	-	-	-	-	$4.5x10^1$	$<0.2x10^0$
Handling/sorting table	-	-	-	-	$6.5x10^1$	$5.0x10^1$
Side of packing area	-	-	-	-	$<0.2x10^0$	$2.8x10^2$

(-) Samples absent

TABLE 46

AIRBORNE MICROBIAL LEVELS, TEMPERATURE AND HUMIDITY LEVELS

Sample Site	Air Sample Count			Temp (°C)	Relative Humidity (%)
	Day 1 am	Day 1 pm	Day 5		
Air vent directed onto conveyor	2	15	7	17.7	38.0
Air vent general area	2	7	5	15.5	41.4
Air bed	384	384	2	24.4	34.7
Air vent onto packing	7	10	10	14.8	43.7
Outside	9	8	-	-	-

TABLE 47 - BIOFILM DEVELOPMENT ON SURFACES

Coupon Position	Time (days)	Biofilm present	Microcolonies present	Occasional cells present	Product debris
Guide bar for air bed	1	x	x	x	x
	3	x	x	x	x
	5	x	x	x	x

TABLE 48 - IDENTIFICATION OF ISOLATES

Area	Microorganisms
Stainless steel surfaces of packing area	*Aeromonas hydrophila* *Acinetobacter calcoaceticus* *Enterobacter agglomerans* *Enterococcus faecium* *Bacillus* sp.

FACTORY 17

This factory produced dry flavourings and spices. Table 49 shows the surface and microbial levels before and after cleaning, and the results show that after cleaning high levels and in all but one case higher than initial levels of microorganisms were detected. In contrast, the ATP levels were generally reduced by cleaning. These results highlight the differences between ATP and TVC analysis. The reduction in ATP levels reflects removal of product debris only from the surfaces as the TVC results are actually higher after cleaning. This is probably due to the spread of contamination by the cleaning regime, so that microorganisms are carried in aerosols or with dust particles to the cleaned surfaces. The ATP levels are generally high even after cleaning but the rim of mixer 2 and packer 3 were particularly high. Coupons were attached to the outlet of the machine packing the flavourings. After 5 hours, occasional microcolonies were observed. This was a somewhat unusual observation, as the significant levels of microorganisms at all the other factory sites could be attributed to the presence of moisture. Whilst microcolony development in dry foods environments was rarely seen, this study indicates that surface attached microcolonies can develop, even in these environments.

TABLE 49 - SURFACE MICROBIAL AND ATP LEVELS BEFORE AND AFTER CLEANING

Sample Site	Before Cleaning		After Cleaning	
	TVC (cfu/cm^2)	ATP (rlu)	TVC (cfu/cm^2)	ATP (rlu)
Packer 1	$2.2x10^0$	20758	$3.0x10^0$	176
Packer 2	$3.4x10^1$	3823	$2.3x10^3$	126
Packer 3	$2.4x10^2$	15845	$1.4x10^5$	6182
Mixer 2 - under rim	$3.4x10^5$	87256	$8.8x10^5$	291970
Mixer 4 - under rim	$3.2x10^5$	-	$1.0x10^6$	-
Storage vessel - inside	$2.4x10^0$	5368	$4.0x10^1$	811
Storage vessel - rim	$1.9x10^5$	-	$4.4x10^0$	-

(-) samples absent

TABLE 50 - BIOFILM DEVELOPMENT ON SURFACES

Coupon Position (5hr)	Biofilm present	Micro-colonies present	Occasional cells present	Product debris
Packing machine outlet	x	(✓)	✓	✓

Summary of the factory data

Table 51 summarises the areas where biofilms were detected. In all cases the development of biofilm was associated with the presence of moisture or condensation. The results show that biofilms were found in a variety of locations; however, the majority of these sites were non-food contact surfaces. Environmental surfaces may be a source of contamination through indirect means such as transfer by vectors (personnel, pests or utensils), cleaning systems or air currents. Previous work has shown that a variety of cleaning techniques generate aerosols of a sufficient size to carry microorganisms over a wide area (Holah *et al* 1990b, 1993a). The most extensive biofilm was found in a blancher extractor system (Factory 1); it is reached almost complete coverage after 120hr. This extractor system was cleaned once per week and as cleaning is the major control of biofilm development there was considerable time available for biofilm formation to occur. The conditions in the extractor system were such that rapid microbial growth would be expected: moisture in the form of steam and condensation, nutrients carried in the air flow from the blancher, and slightly elevated temperatures. The biofilms associated with air extraction units are of concern in food production environments as spoilage and or pathogenic microorganisms in aerosols may gain access to the factory through air intakes.

In excess of 50% coverage was observed on the ledge underneath a mixer, where the organisms on the surface were exposed to intermittent nutrient and water supply through splashing. Biofilm was also found in a steam clean room; this is a common location for extensive surface microflora development due to the elevated temperatures and condensation. The ceiling in a potato processing area showed extensive multilayer microcolony development, although the overall coverage of the surface was patchy probably due to the uneven distribution of condensation droplets. This area had particular problems with condensation and this was probably the major reason for the biofilm development.

Conveyor belting material is particularly difficult to clean, and showed rapid development of a substantial surface population. These surfaces may be product contact surfaces and therefore may be major sources of contamination. Bizzaro *et al* (1990) similarly found high levels of organisms on polyurethane bands in a meat slicing plant (10^5 mesophilic aerobic bacteria per cm^2).

Table 52 shows that biofilms were detected on a small proportion of the coupons attached to various locations in factory environments (6.6%); however, micro-colonies were

observed on one fifth of the coupons. These results show that the incidence of biofilms was low, and that the incidence of microcolony formation was reasonably low in the areas targeted in this study. The areas selected for study were considered to be areas that would promote surface growth and therefore the existence of biofilm generally is lower. Table 53 summarises the areas where micro-colonies were detected. These micro-colonies may develop into substantial biofilms over longer exposure times. Drains and gutters are particularly prone to colonisation as biofilms readily form in flowing systems due to the regular supply of nutrients.

The majority of the surface showing micro-colony development could be attributed to the presence of moisture; however, microcolonies of microorganisms were detected on the surfaces of the dry goods manufacturing equipment of factory 17.

Biofilms often develop in the food industry due to poor practices and poor equipment and factory design features. For example, inadequate ventilation systems result in high levels of humidity and/or the presence of condensation on surfaces so that microorganisms can readily grow and proliferate. Holah and Thorpe (1990) demonstrated the importance of surface topography as bacteria attached in pits and crevices were very difficult to remove due to poor penetration of cleaning chemicals. Flemming (1991) listed the factors affecting biofilm potential in industrial water systems and Mattila-Sandholm and Wirtanen (1992) suggested that these were equally applicable to the food processing industry. Flemming (1991) suggests that the following have high biofouling potential: extended piping; dead legs; rough surfaces; non-disinfected holding tanks; elevated temperatures; high concentrations of nutrients and cells; intermittent operation and poor access to surfaces. In contrast the following were suggested to have low biofouling potential: low temperatures; smooth surfaces; early and preventive cleaning; good access to surfaces etc.

Table 54 shows the types of organisms present on the surfaces in the different processing environments and Table 55 shows the frequency of the isolated genera and general groups of microbes. The organisms were identified using the Vitek P60 database which is designed for clinical isolates, and this may be the reason for only 66.4% of the isolates being successfully identified. The figures in Table 55 (a) are percentages of the different genera in the identified isolates and Table 55 (b) shows the percentage of the major groups of microbes.

Of the identified isolates, 79% were Gram negative rods; there were similar proportions of oxidase positive and oxidase negative organisms. (ie 50:50).

Gram negative (oxidase positive) rods are the most common spoilage organisms of chilled foods, and rapidly dominate the microflora of fresh chilled foods such as meat, poultry, fish and dairy (Walker, 1992). Within this group of organisms, the genus *Pseudomonas* is most common, although other genera include *Aeromonas, Acinetobacter, Flavobacterium, Moraxella,* and *Vibrio* species. Table 55 (a) shows that pseudomonads were the most commonly isolated organisms. These organisms are common in the environment, particularly water, and therefore may easily contaminate food. These organisms may grow on inadequately cleaned surfaces of food processing plant or equipment and so contaminate foods. Pseudomonads in particular have been shown in other studies to readily attach to stainless steel, forming extensive biofilms (Mattila-Sandholm and Wirtanen, 1992; Spenceley, 1993). Gram negative (oxidase positive) rods may spoil products by the production of diffusible pigments, slime material on surfaces and enzymes which result in rots, off flavours and odours. Spoilage is most rapid in proteinaceous foods such as red meats, poultry, fish and dairy products. Table 54 shows the isolates identified from the environment of particular product types, for example, *Pseudomonas* and *Aeromonas* species were isolated from the cheese production area. This group is well adapted to grow at chill temperatures, and may explain the prevalence of these microbes in chilled environments such as in salad production *(Flavobacterium, Aeromonas, Acinetobacter* and *Pseudomonas* species).

The Gram negative (oxidase negative) group comprises coliform/enteric bacteria including *Citrobacter, Escherichia, Enterobacter, Klebsiella, Proteus* and *Serratia.* Generally this group is less well adapted to growth at temperatures less than 5-10°C but may dominate the flora at temperatures of 8-15°C. These microbes are widely distributed in the environment, including animals. This group of microorganisms is often used as an indicator of inadequate processing or post process contamination. The figures in Table 1.54 (a) show that *Enterobacter* species were one of the more commonly isolated species.

The Gram positive coccoid organisms (*Staphylococcus* spp) comprised 8.6% of the identified isolates. Staphylococci are associated with human skin, nasal and auricular cavities and other skin niches. Skin flakes with associated microbes would therefore be expected to be found in any environments where people are working.

Gram positive (spore forming) rods were found in the cheese, beans, meat (pasta and pizza) and biscuit production factories. *Bacillus* species are common environmental organisms.

The following potentially pathogenic microorganisms were isolated: *Aeromonas*

hydrophila, Staphylococcus aureus, Vibrio fluvialis.

Aeromonas hydrophila was isolated from the cheese processing, salad production and biscuit production factories. This organism has been implicated in foodborne disease; however, it is common in the environment, particularly water, and may therefore easily contaminate food and the food processing environment. *A.hydrophila* is considered to be heat sensitive and so may be readily eliminated from foods.

Vibrio species are prevalent in aquatic environments and therefore foods, particularly seafoods, harvested from these areas may be contaminated. Two *Vibrio* species were isolated in this study, and *Vibrio fluvialis* has been associated with foodborne disease. This organism was isolated from the pea processing environment and may have been carried in on the peas.

Cleaning and disinfection systems are the major control of the hygiene of surfaces. The efficiency of the factory cleaning and disinfection regimes was variable. In some cases the cleaning and disinfection programme effectively reduced both the ATP and TVC. In other cases the ATP values were significantly lower after cleaning and disinfection, although the TVC remained unchanged or was higher after cleaning. These results suggest that the cleaning programme had reduced the level of product soil present on the surfaces whilst the levels of microbes were similar or higher. The higher levels of microorganisms after cleaning and disinfection may actually be a consequence of the cleaning techniques and sequence of the cleaning programme in the factory. High pressure cleaning, for example, generates aerosols that can transfer microbes to disinfected equipment surfaces.

TABLE 51 - SUMMARY OF AREAS WHERE BIOFILMS WERE DETECTED

Area	Exposure Time (hr)	Count/cm²	% Coverage	Product	Factory
Waste can area	24	$\geq 3.5 \times 10^7$	15.0	Canned product	1
Blancher extractor	24	$\geq 4.7 \times 10^7$	26.5	Canned products	1
	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	10
Ceiling	24	5.5×10^5	3.6	Potato	8
Conveyor	1.5	4.6×10^6	11.3	Cod cakes	8
Steam clean room	16	$\geq 1.4 \times 10^7$	20.2	-	15
Wall in rack washing area	6	-	-	Poultry products	14
Inspection belt guard	48	-	-	Peas	14

TABLE 52 - SUMMARY OF THE SURFACE MICROBIAL LEVELS ON ALL THE STAINLESS STEEL COUPONS

	% of Coupons (n = 120)
Biofilm	6.6
Microcolonies	20.8
Occasional cells	37.5
Product only	35.1

TABLE 53

SUMMARY OF THE AREAS WHERE MICROCOLONIES WERE DETECTED

Area	Exposure Time (hr)	Product	Factory
Egg glaze machine	6	Various	5
Drain	8	Fish	9
Drain	10	Salads	12
Bowl chopper guard	6	Poultry	4
Ceiling	6	Poultry	4
Guide rail	6	Poultry	4
Side of conveyor	5	Various	6
Side of mixer	5	Various	6
Inspection belt guard	24	Peas	14
Mezzanine chute outlet	48	Peas	14
Inspection belt guard	49	Peas	14
Packing machine outlet	5	Flavourings	17

TABLE 54 - SUMMARY OF THE TYPES OF ORGANISMS IDENTIFIED FROM DIFFERENT PROCESSING ENVIRONMENTS

Product Type (Factory No.)	Organisms Identified
Cheese (Factory 11)	*Bacillus asacchorolytic* *Aeromonas hydrophila* *Staphylococcus warnerei* *Pseudomonas pickettii* *Pseudomonas aeruginosa*
Fish (Factory 9)	*Citrobacter freundii* *Enterobacter agglomerans* *Staphylococcus auricularis* *Pseudomonas fluorescens* *Proteus vulgaris* *Kluyvera* species *Pseudomonas paucimobilis*
Meat - poultry (Factory 4)	*Hafnia alvei* *Serratia liquefaciens* *Staphylococcus sciuri* *Serratia marcescens*
Salads Site 1 (Factory 12)	*Agrobacterium tumefaciens* *Hafnia alvei* *Flavobacterium indologines* *Aeromonas hydrophilia*
Salads Site 2 (Factory 13)	*Acinetobacter calcoaceticus* *Klebsiella pneumoniae* *Serratia plymuthica* *Pseudomonas fluorescens*

Salad Site 2 cont.	*Enterobacter agglomerans* *Pseudomonas vesicularis* *Kluyvera* species *Citrobacter freundii* *Pseudomonas aeruginosa*
Peas (Factory 14)	*Klebsiella pneumoniae* *Pseudomonas fluorescens* *Vibrio fluvialis* *Enterobacter agglomerans* *Trichosporon beigelii*
Potato products (Factory 8)	*Flavobacterium* species *Pseudomonas fluorescens* Yeast *Providencia rettgeri*
Beans - Site 1 (Factory 1)	*Yersinia pseudotuberculosis* *Agrobacterium tumefaciens* *Escherichia coli* *Enterobacter agglomerans* *Pseudomonas acidovorans* *Bacillus asaccharolytic* *Pseudomonas paucimobilus*
Beans - Site 2 (Factory 1)	*Pseudomonas paucimobilis* *Flavobacterium indologines* *Pseudomonas versicularis* *Pasteurella haemolytica* *Comamonas acidovorans* *Bacillus* species *Pseudomonas picketti* *Flavobacterium* species *Vibrio alginolyticus* *Sphingobacterium multivorum* *(Flavobacterium multivorum)*

Meat, Pasta, Pizza (Factory 5)	*Staphylococcus saprophyticus* *Staphylococcus warneri* *Pseudomonas vesicularis* *Bacillus* species
Protein substitute (Factory 10)	*Pseudomonas fluorescens* *Xanthomonas maltophilia* *Staphylococcus warneri*
Pizza (Factory 6)	*Enterobacter agglomerans* *Acinetobacter calcoaceticus* *Pseudomonas paucimobilis* *Serratia liquefaciens*
Biscuits (Factories 15 + 16)	*Pseudomonas fluorescens* *Flavobacterium indologenes* *Acinetobacter calcoaceticus* *Staphylococcus saprophyticus* *Aeromonas hydrophila* *Enterobacter agglomerans* *Enterococcus faecium* *Bacillus* species

TABLE 55 - SUMMARY OF THE FREQUENCY OF GENERA AMONG IDENTIFIED ISOLATES

(a)

Genera	Percentage (n = 78)
Pseudomonas	23.0
Staphylococcus	8.6
Enterobacter	8.6
Flavobacterium	7.7
Acinetobacter	7.7
Bacillus	6.5
Serratia	5.1
Klebsiella	5.1
Aeromonas	3.8
Vibrio	2.4
Citrobacter	2.4
Kluyvera	2.4
Agrobacterium	2.4
Hafnia	2.4
Providencia	1.2
Escherichia	1.2
Pasteurella	1.2
Proteus	1.2
Yersinia	1.2
Trichosporon	1.2

(b)

Organism Group	Percentage (n = 78)
Gram negative rods - oxidase positive	40.5
Gram negative rods - oxidase negative	38.5
Gram positive cocci	8.6
Gram positive rods	6.5
Yeasts	1.2

CONCLUSIONS

The results presented show that biofilms may develop on surfaces in the food processing environment, although the incidence of thick multilayer biofilms was low (<6.6% of coupons). Microcolony development was found on 20% of the coupons attached to the food processing environment; the majority of these surfaces were environmental surfaces. These microcolonies may develop into substantial biofilms over longer exposure, particularly on environmental surfaces which are cleaned less frequently than product contact surfaces. In fact, biofilms and substantial microcolony formation were only found on infrequently cleaned surfaces, emphasizing the importance of cleaning and disinfection. Environmental surfaces may be a source of contamination through indirect means such as transfer by personnel, pests, utensils, cleaning systems or air currents.

The environmental factor associated with biofilm development in all cases was the presence of moisture, and therefore the control of water, steam, condensation and humidity is critical to prevention of biofilm development. Microcolony formation was also attributable to the presence of moisture in the majority of cases. Slightly higher than ambient temperatures were also associated with particularly extensive biofilm growth, but it is not clear whether chill temperatures significantly impede biofilm development.

The organisms present on the surface fell mainly into two groups: Gram negative (oxidase positive) spoilage organisms and Gram negative (oxidase negative) enterics and coliforms. The surfaces of the processing environment may therefore be a major source of spoilage organisms. Pseudomonads were particularly prevalent on the surfaces. Pathogenic microorganisms were identified from relatively few surfaces; however, surfaces may be a source of pathogenic microbes (bacteria may preferentially attach to particular surface types, for example product surface as opposed to stainless steel surface).

Cleaning and disinfection systems are the major control of the hygiene of surfaces. The efficiency of the factory cleaning and disinfection regimes was variable. The efficiency of the cleaning and disinfection programme can be rapidly assessed using ATP bioluminescence techniques, and surfaces recleaned if necessary before production restarts. It is important to note that relatively high numbers of microbes may be present on a product-free surface with a low ATP reading.

The importance of equipment hygienic design and hygienic processing was apparent. Poorly designed equipment such as conveyor belting, which is particularly difficult to clean, was consistently found to harbour high levels of microorganisms even after

cleaning. Inadequate ventilation systems resulted in high levels of humidity and the presence of condensation on surfaces, which promoted the growth of microorganisms.

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