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BACTERIOLOGICAL CONTROL OF FOOD EQUIPMENT BY CLEANING SYSTEMS

A thesis submitted in partial fulfillment of

the requirements for the degree of

Doctor of Philosophy

at the

University of Waikato

by

DAVID GEORGE DUNSMORE

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1978

ADDENDA

Soak Cleaning

When cleaning solutions are applied cold the bactericidal and detergency benefits of heat are discarded. These disadvantages can only be overcome by applying the cleaning solutions at higher concentrations or with longer contact times (soaking).

It is possible to use soak cleaning on pipeline milking machines, but there are two disadvantages. A number of modifications must be made to the machine to ensure that the solutions are maintained in contact with the surfaces for the full intercycle period. Also, the economic gains, achieved by eliminating the heat requirement of cleaning solutions, are offset by the increased costs incurred in the higher concentrations of detergents and sanitizers which are required.

If these costs can be reduced by re-using the detergent for a number of cleaning cycles, then soak cleaning can become a potentially valuable method of cleaning milking machines. It is extremely important that a detergent is not re-used to the point where it is ineffective. This limit, and adequate safety margins, can only be determined by field trial. Unfortunately, when the field trial described in Section 2.6 was designed, there was insufficient knowledge available on soak-cleaning and re-use to permit these systems to be incorporated. Consequently, the trial was conducted using the turbulent flow method of solution application.

Selection of Dosing Organisms

At the commencement of this work it was decided that to ensure a bacteriological soiling cycle occurred, constant numbers of contaminating bacteria would be allowed to interact with the test surfaces. The easiest way to have introduced contaminating bacteria would have been to use raw milk, but with the disadvantage of its highly variable natural flora. The alternative was to try and introduce a limited and controlled number of organisms to the milk prior to each soiling cycle. This latter approach was selected as the most appropriate technique in this, the first use of bacterial assessment of cleaning efficiency by simulator.

This technique has provided valuable information under the limited situations in which it has been tested. Although the selection of organisms used was based on their resistance to components of the cleaning system, the failure of all three species to grow during intercycle periods suggests there could have been a more successful selection of dosing organisms. As a consequence no active community of bacteria developed on the test surfaces as might have been expected. In future, selection of organisms could be made from a soil in equilibrium with a cleaning regime, or a natural flora could be allowed to develop from raw milk. The inclusion of "harbourages" in the substrates to provide more appropriate conditions for soil accumulation and moisture retention would also aid in the establishment of an active community.

Application of Results from the Soak Simulator

The soak-cleaning simulator has proved valuable for establishing the principles of cleaning system performance. However, the results of these studies can only be practically applied to equipment that is soak-cleaned.

If a cleaning system is required for flow-cleaned milking machines the development must be conducted on a simulator which applies the cleaning solutions turbulently. The simulator described in this thesis has now been modified for that purpose.

ERRATA

Please note the following corrections:

Page	Comment
1.5 - 19	Read "Strumbo" for "Stumbo"
1.5 - 33	Read "Adaptation" for "adaption"
1.5 - 47	Read "Twomey 1966" for "Twomey, 1966"
1.5 - 57	Read "Clegg" for "Cleg"
1.5 - 76	Read "Compared with" for "compared to" Read "bases" for "basis"
1.5 - 77	Read "alteration" for "alteratian"
1.5 - 80	Read "Thomas & Thomas 1977, II" for "Thomas & Thomas 1977b"
1.5 - 93	Read "to" for "ot"
1.5 - 95	Read "Ministry of Agriculture, Fisheries and Food" for "MAFF"
1.5 - 96	Read "information" for "nformation"
2.3 - 23	Read "attached to" for "colonised"
2.3 - 25	Read "effective" for "totally effective"
2.4 - 1	Read "et al." for "*et al*"
2.4 - 12	Read "none was" for "none were"
2.6 - 4	Read "bacteriological" for "keeping"
2.6 - 8	Read "consisted" for "con sited"
2.6 - 9	Table 2.6 - 3 - Note that the microbacteria are included with the coryneforms which were not detected in this experiment.
3.1 - 16	Read "showed that a chlorine sanitizer" for "showed that, a chlorine sanitizer"
3.1 - 19	Read "compared with" for "compared to"
3.1 - 22	Replace 3.with "3. All components of the cleaning system - rinses, detergents and sanitizers - may reduce the numbers of viable bacteria by removal or killing. The relative importance of these two mechanisms should be studied under field conditions".

3.2 - 2	Read "experiments showed that none" for "experiments showed than none".
3.2 - 3	Replace "the major concentration.....teat cups", with "the bacteria in the milk may originate in the udder or from contaminated teat skin".
4. - 14	Read "J. appl. Bacteriol" for "J. Appl. Bacteriol".
4. - 28	Read "N.Z. J. Dairy Technol" for "N.Z. J. dairy Technol
4. - 29	Insert "Johnson, M.C., J. J. Bozzola, I. L. Shechmeiste and I. L. Shklair. 1977. Biochemical study of the relationship of extra cellular glucan to adherence and cariogenicity in *Streptococcus mutans* and an extracellular polysaccharide mutant. J. Bacteriol. 129: 351-357".
4. - 37	Insert "McDougall, J. 1969. Microbiological aspects of metal corrosion. Australas. Corros. Eng. 13: 13-16.

ABSTRACT

This study was undertaken to examine the performance of cleaning systems applied to food equipment. The contribution by the components of those systems to the performance of the total system was determined.

A cleaning simulator was constructed in which test pieces in 4 test cells were subjected to 25 to 35 cycles, each of 12h. Each cycle the test pieces were: soiled for 45 min. in pasteurised milk (at 30°C) dosed with 10^6 bacteria/ml of Streptococcus faecalis, Escherichia coli or Enterobacter aerogenes; serially treated with the rinses, detergents and sanitizers of the experimental cleaning system; and left for 9.5h intercycle (rest) period. The changes in the soil mass with time on stainless steel, rubber and glass test pieces were determined gravimetrically. The changes in the numbers of the dosed and non-dosed organisms on the surfaces, after components of the cleaning system had been applied, were determined by a swab-recovery/plate-counting system. The surfaces were also examined by scanning electron microscopy. Four experiments were performed using this technique.

A field experiment examined the performance of 5 cleaning systems over experimental periods of 5 weeks on 15 milking machines.

The nature of the changes in the performance of the cleaning system over time were determined. Both detergents and sanitizers could, under varying conditions, contribute significantly to the bacteriological performance achieved by the system. A sanitizer applied for the complete intercycle period was more efficient than those applied for short contact-times. A post-wash sanitizer was slightly more efficient than a sanitizer applied before milking.

The cycle of bacterial deposition onto the equipment surfaces, attachment, depletion by the cleaning system, growth during the intercycle period, and subsequent contamination of the milk at the next milking was studied. All three dose organisms attached actively, and were then largely removed by the cleaning system. Despite there being a large amount of soil on the surfaces of the test pieces, the intercycle conditions (30°C at up to 90% RH for 9.5h) were insufficient for growth. The effect of the amount of soil on the surface on the ability of bacteria to survive the application of a system or system component was examined. The minimum soil levels necessary for an organism to survive a treatment (Total Effect Maximum) and the minimum soil necessary for all organisms to survive a treatment (Zero Effect Minimum) were determined for systems and system components.

The data was used to discuss the principles to be followed in designing a cleaning system. The principles were applied to the design of a system to clean a milking machine with cold solutions by soak techniques.

PREFACE

There is continuing pressure from food-consumers and public health authorities for improvement in the hygienic quality of foods. Having handled an extremely delicate product for many years, the dairy industry has developed significantly more scientific and technological information on food hygiene than any other section of the food industry. Most of the work has concentrated on the cleaning of milking machines and other farm equipment, as these are potentially the greatest contributors to the contamination of milk. This knowledge serves as a firm foundation for further studies in dairy hygiene. The information obtained in such studies can be applied to the remainder of the food industry.

The developed countries have recently experienced an energy crisis. Intensive dairying is spreading throughout the underdeveloped countries. Both of these factors create a demand for simplified techniques which clean equipment effectively, but do not require great amounts of thermal or kinetic energy.

Dairy equipment is maintained in a hygienic condition by cleaning systems. Any improvement in hygiene requires an understanding of the factors affecting the performance of those systems. This information was not available at the commencement of this study.

The work described in this thesis was undertaken to investigate the following topics:

1. The contribution of each component of the cleaning system to the performance of the total system - assessed in terms of the control of soil accumulation and bacterial development on equipment surfaces;
2. The nature of the changes in the performance of cleaning systems

with time;

3. The inter-relationships between the physical, chemical and biological characteristics of cleaning systems;
4. The cyclic changes which take place in the bacterial population present on equipment surfaces, commencing with deposition from milk and finishing with contamination of the milk; and
5. The principles underlying the design of cleaning systems.

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The construction of the simulator was a very large task in itself. I would like to thank Mr D. Jay for his construction and maintenance of the very valuable programmer, Mr D. Westwood for a great construction job and Mr R. Harrison of the National Dairy Association for the magnificent stainless steel apparatus.

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1

THE SELECTION AND PERFORMANCE OF CLEANING SYSTEMS FOR FOOD EQUIPMENT

1.1 WHAT IS A CLEANING SYSTEM?

1.1.1: THE CLEANING TASK

It is imperative that all product-contact surfaces of food-handling equipment be clean in order to prevent any physical, chemical or biological contamination of the product. The equipment surfaces are maintained in this hygienic condition by the application of a "cleaning system". This sytem includes a specific sequence of rinses, detergents and sanitizers applied by defined physical techniques,and complemented by particular conditions between uses of the equipment.

Swartling (1959) outlined the following three principles of a cleaning system:

1. Removal of food residues that can serve as nutrients for any bacteria remaining on the surface;
2. Destruction of any bacteria not killed or physically removed together with the food residues;
3. Storage of utensils under conditions which discourage or prevent the growth of any surviving organisms.

To this list the following must be added;

4. Removal of any cleaning solution residues that may contaminate the product.

In practice,most attention is given to the cleaning and sanitizing operations within the cleaning system. These are complementary pro-

cesses; neither alone can achieve the desired result. Classically, the cleaning and sanitizing functions can be performed by separate components of the cleaning system or by single components. However, this classification into "cleaning" and "sanitizing" is quite arbitrary as all cleaning system components perform both some cleaning and some sanitizing function (Ashton, 1961; Davis, 1956a). For this reason some workers have preferred to use the term "cleanse" to denote the combined cleaning and sanitizing operations (Clegg 1962; Davis 1956a).

An example of a conventional system for cleaning milking machines is presented in Table 1.1-1, (Scott et al. 1962). This system is applied after each milking (every 12h) and contains the following components: post-milk rinse, detergent wash, post-detergent rinse, and pre-milk sanitizer. This number of operations may be extended or reduced, some functions may be combined, and the time at which they are applied may be altered. Also, the chemical materials used can be selected from a very wide range of possible classes and formulations within each class. As a result the theoretical number of potential cleaning systems is almost infinite. In practice, however, the actual number of systems used is quite small as the requirements of performing efficiently and reliably, while being both practical to apply and economical in use limit the selection greatly.

Cleaning systems can be divided into four classes according to whether heat is used as a sanitizing agent, and whether the main, post-product wash is performed with an acidic or alkaline solution. The classes are:

1. Heat sanitizing, acid wash;
2. Heat sanitizing, alkaline wash;

Table 1.1.1: An example of a cleaning system applied to milking machines

Time of operation	Time after milking (h)	Operation	Concentrate	Temperature °C	Application method
Milking A	-	-	-	-	-
Post-milking 1	0.00	Rinse	-	Ambient	Flush
2	0.10	Wash	Alkaline detergent	60-70°C	Flush or recirculate
3	0.20	Rinse	-	85°C	Flush
Intercycle	0.30	Drain	-	Ambient	-
Pre-milking	4.50	Sanitizer	Iodophor	Ambient	Flush
Milking B	10.00				

3. Sanitizing without heat, acid wash; and

4. Sanitizing without heat, alkaline wash.

These classes may be further subdivided, if required, depending on whether cleaning and sanitizing are performed as separate operations or combined.

What level of performance is required of a cleaning system? Davis (1956a) defined the "minimum efficiency level" as that level of plant operations which can be relied upon to produce products of sufficient quality to always meet any quality criteria by a safe margin. The minimum efficiency level is affected by the quality of the product as the product enters a piece of equipment and also by the treatment of the product after passing through the equipment. However, the factor most affecting the minimum efficiency level is the performance of the cleaning system.

ACTIONS OF THE CLEANING SYSTEM

The actions of the cleaning system are very complex, with various physical, chemical and biological processes occurring simultaneously. These actions will be summarised here, and discussed in full (with references) in Sections 1.4 and 1.5. The actions will be discussed using the heat-sanitizing, alkali-wash system of Scott et al. (1962) as an example (Table 1.1-1).

MILKING MACHINE DESIGNS

There are two main designs of milking machines; the "bucket" machine and the "pipeline" machine. During milking, the teat cups (I) of the bucket machine (Figure 1.1-1) are placed on the teats of the cow and the milk is drawn through the long milk tube (H) to the bucket (K) by the vacuum supplied through the vacuum line (A). After

Figure 1.1-1: Cleaning arrangement for bucket milking unit

Key:
- A Vacuum line
- B Stall tap
- C Air tube
- D Pulsator
- E Pulse line
- F Air tube
- G Restrictor
- H Long milk tube
- I Cluster (teat cups)
- J Pail holding cleaning solution
- K Milking bucket

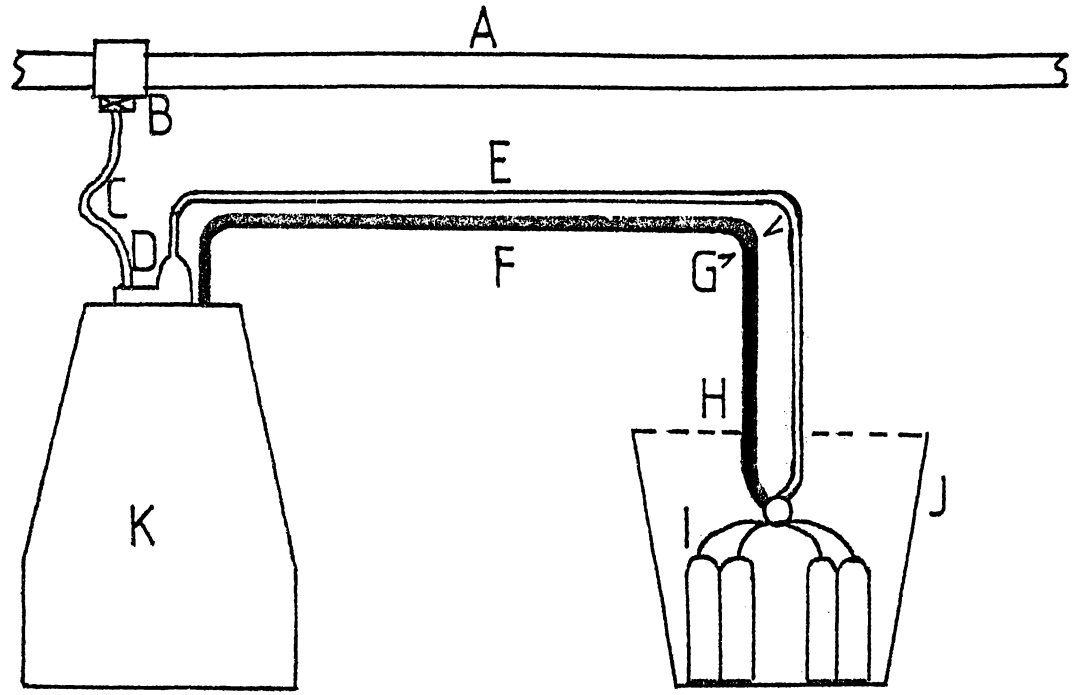

A
B
C
D
E
F
G
H
I
J
K

Figure 1.1-2: Solution flow through a flush-cleaned pipeline milking machine

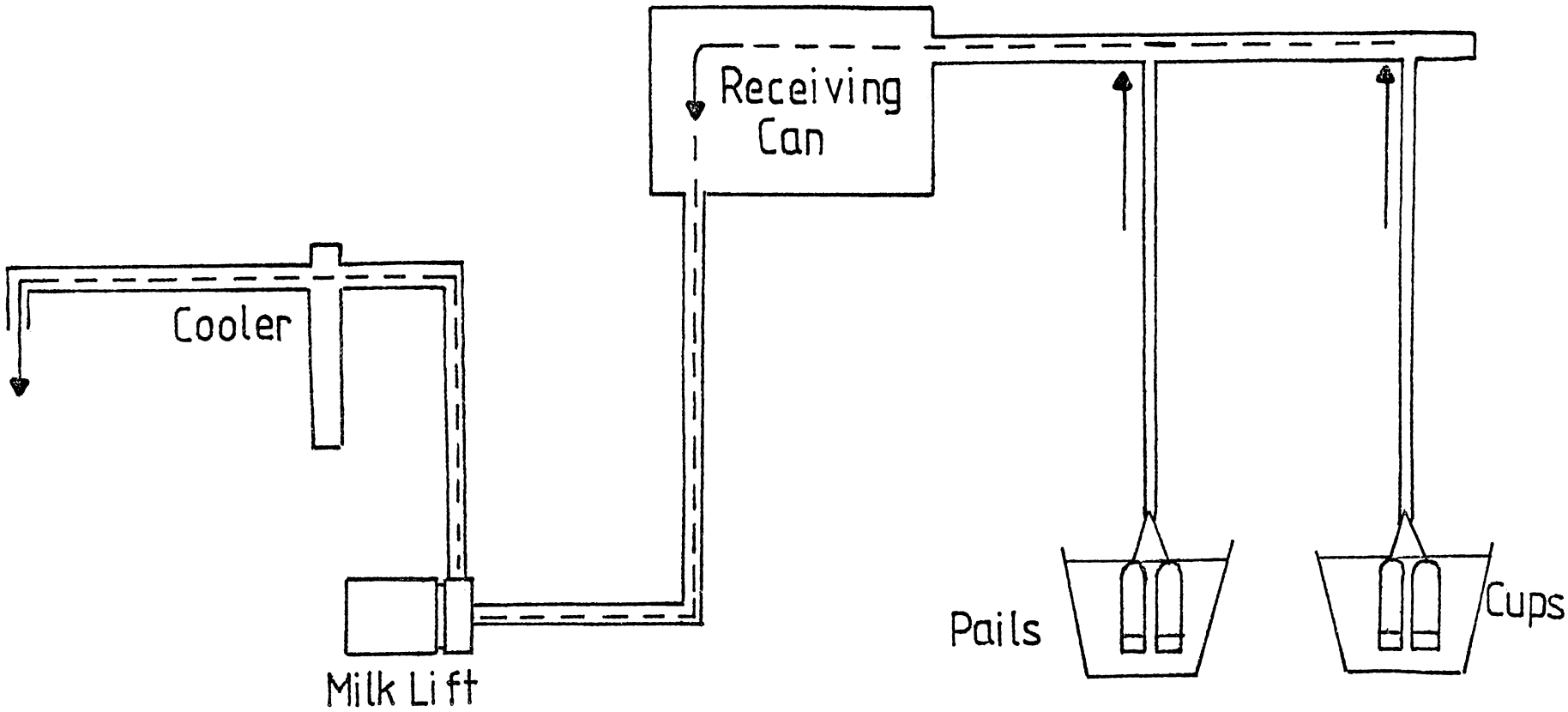

Receiving
Can
Cooler
Milk Lift
Pails
Cups

Figure 1.1-3: Solution flow through a circulation-cleaned pipeline milking machine

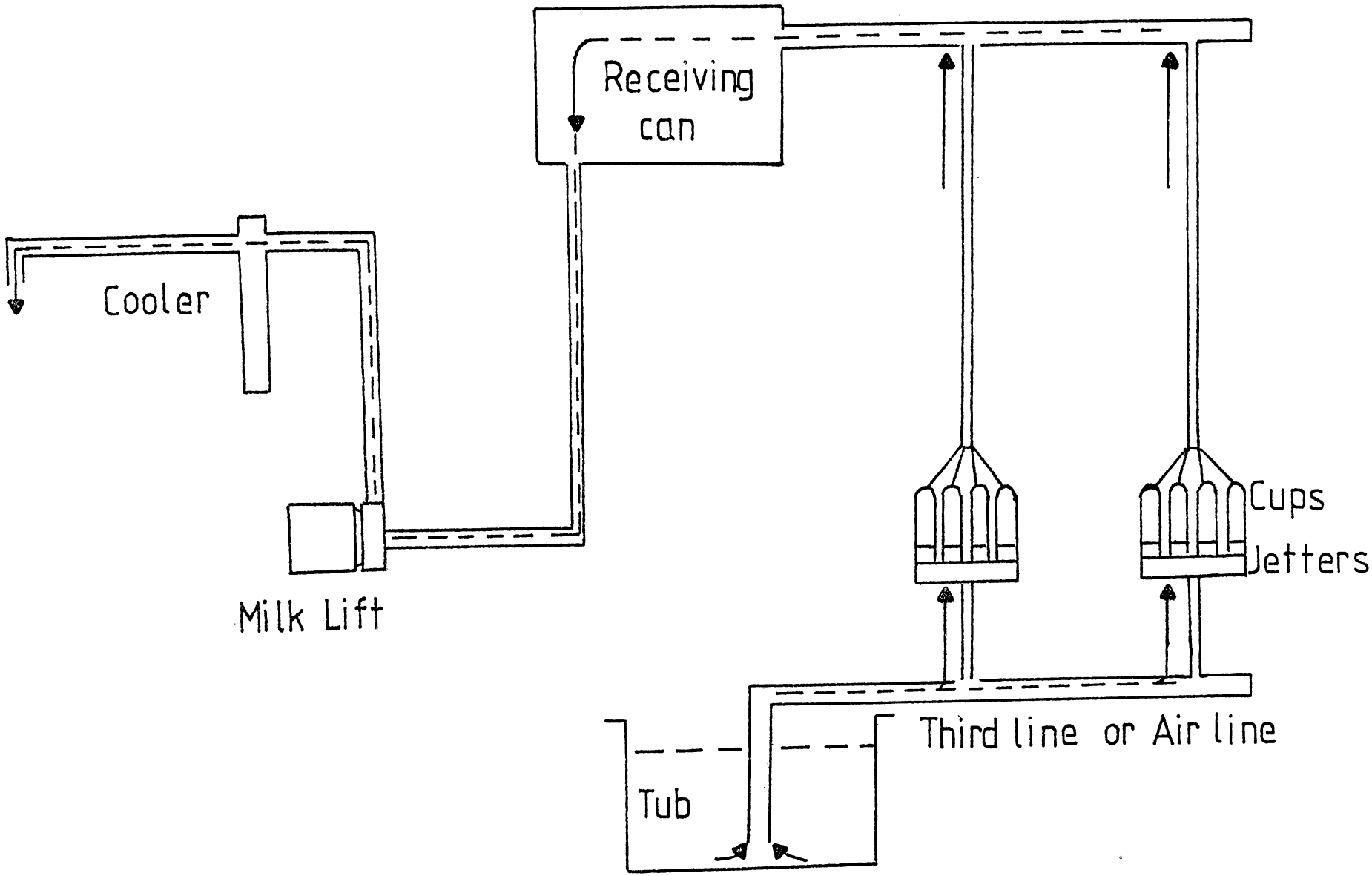
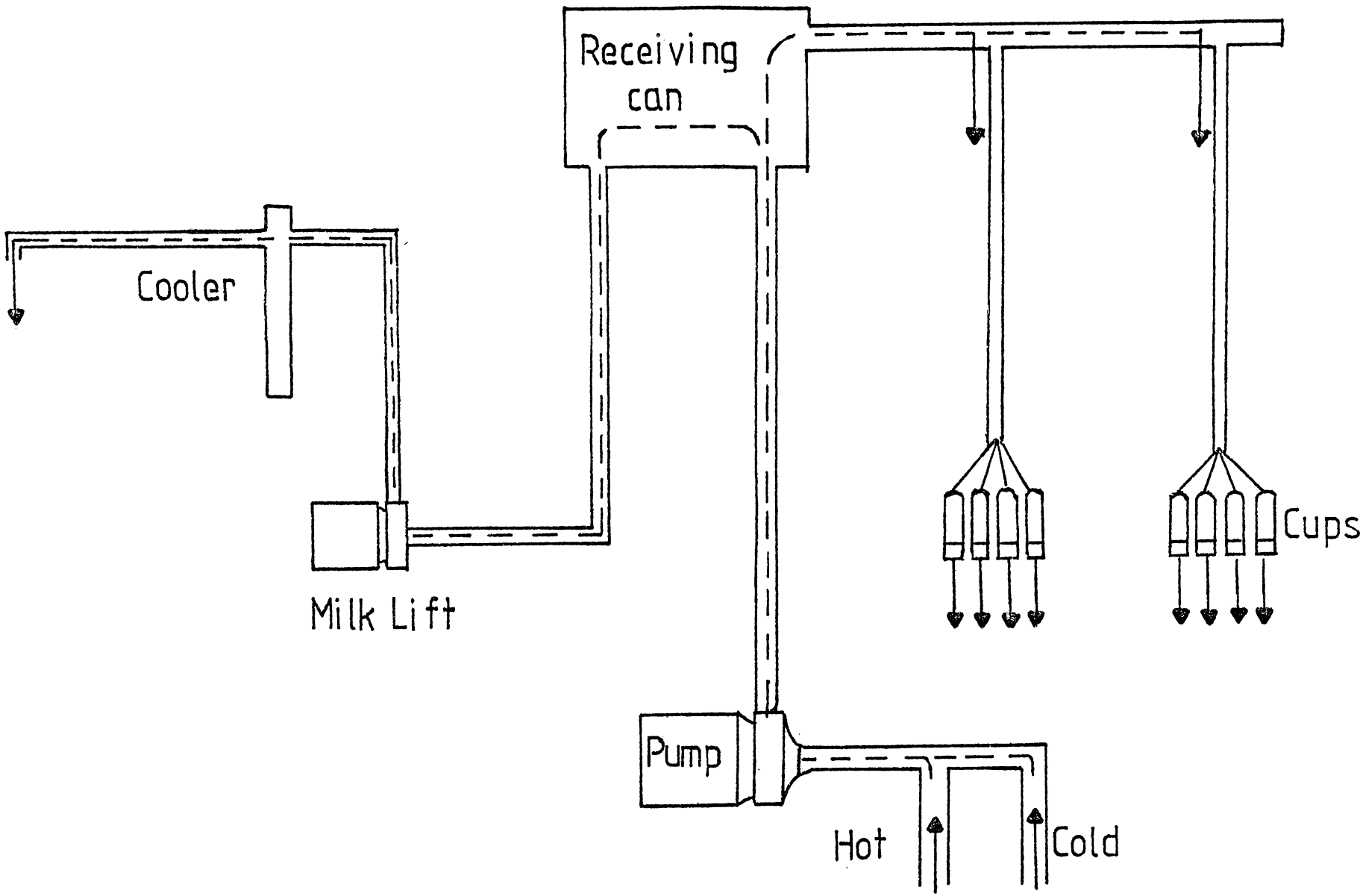

Receiving
can
Cooler
Milk Lift
Cups
Jetters
Third line or Air line
Tub

Figure 1.1-4: Solution flow through a pipeline milking machine cleaned by the reverse-flow technique


Receiving
can
Cooler
Milk Lift
Cups
Pump
Hot
Cold

milking a number of cows the bucket will be filled, and it will be removed and emptied into a storage vessel, being replaced by an empty bucket. For cleaning, the cleaning solution is prepared in a pail (J), drawn through the teat cups to the bucket and then discarded.

During milking, milk from the teat cups on a pipeline machine (Figures 1.1.2-4) is drawn through to the receiving can by vacuum and then pumped to the storage vat through a cooler. When the machine is "flush-cleaned" the solutions are prepared in pails, and drawn through the teat-cups by vacuum in the same way as the milk (Figure 1.1-2). The solutions are then pumped through the cooler to waste. With "circulation-cleaning" the solutions are prepared in a tub and supplied to the teat cups through jetters (Figure 1.1-3). Sometimes recirculation-cleaning is achieved by returning the exhausted solution to the tub. With "reverse-flow-cleaning" the solutions are pumped positively in through the receiving can and out by both the teat cups and cooler (Figure 1.1-4).

PHYSICAL PROCESSES

After milking, a milk residue is left on the product-contact surfaces. Some of this will be adsorbed onto the surface, but most will merely lie on the surfaces unattached. The application of the post-milk rinse flushes away the unattached residue. The detergent is then applied and it removes and flushes away most of the adsorbed milk residue, the extent of removal depending on the formulation, temperature and flow conditions of the solution. As the detergent is drained away it leaves a small amount of milk residue and detergent chemicals on the surface, which has reached a temperature of $\sim 60^{\circ}C$. The post-detergent rinse is then applied,which flushes away the mix-

ture of loosely-attached milk soil and detergent chemicals, while raising the temperature of the equipment to about 80°C. After this rinse is drained, the equipment surfaces, and any remaining soil, dry and then cool to ambient temperature between milkings. Immediately prior to the next milking, the pre-milking sanitizer is flushed through the equipment, removing some of the milk soil and wetting the surfaces before the milk is passed through. The actions of the physical forces are discussed in detail in Section 1.4-2 on the physics of cleaning.

CHEMICAL PROCESSES

Milk is a stable emulsion containing both acid-soluble (mineral) and alkali-soluble fractions (primarily fat and protein). The application of the post-milking rinse disturbs the stability of the milk emulsion, disperses most of the milk components in the rinse solution and flushes them away. The components of the alkaline detergent which follows the rinse detach most of the adsorbed organic fraction of the soil (fat and protein), and degrade macro-molecules to smaller, more soluble, particles. Most of these solubilised soil particles are then flushed away with the detergent solution, leaving a mineral type of soil on the surfaces. The post-detergent rinse flushes away the loosely-attached detergent-soil complexes and any residual detergent reagents. In the period between milkings, the soil remaining on the surface dries out and becomes more adherent. The pre-milking rinse contains iodophor as a sanitizer. This has acidic-detergent properties which permit it to solubilize and remove much of the mineral soil that has been left by the alkaline detergent. The sanitizer flushes away any dissolved soil, leaving any surface-active components adhering to the

soil and the surfaces, along with a film of liquid sanitizer solution.

These actions of the detergent components will be described in detail in Section 1.4-1.

BIOLOGICAL PROCESSES

The only groups of organisms which are of importance in dairy equipment hygiene are the bacteria. These may be present in the milk, the cleaning solutions, or the atmosphere. As milk is an ideal growth medium for bacteria, any residues on the surfaces of the milking machine will be colonised, and at the subsequent milking some of the bacteria will contaminate the milk passing over the equipment surfaces.

The post-milking rinse flushes away most of the milk residues and thereby removes many of the bacteria they contain. The detergent which follows at 60°C will further reduce the numbers of bacteria by physically removing them, but this will be complemented by the bactericidal effects of the detergent reagents and temperature on some of the organisms. Those bacteria which remain on the surfaces will be subjected to further depletion by the final flushing rinse, the temperatures of which (80°C) will kill most of the non-thermoduric organisms. After the final rinse the soil and equipment surfaces dry out, leaving an atmosphere which may be insufficiently humid to support growth of any surviving bacteria. The pre-milking iodophor sanitizer further depletes the numbers of organisms, by removing some through detergent action on the mineral soil, and by bactericidal effect on any organisms with which it remains in contact for sufficient time. The soil remaining on the surfaces during the sanitizing operation can protect the bacteria both chemically, by reacting with and thereby inactivating the sanitizing agent, or by physically protecting organisms in such a

way that they do not come into contact with the sanitizing agent. The viable organisms which remain on the surfaces of the equipment after the final pre-milking sanitizing may then contaminate the milk. The sanitizing processes are discussed in detail in Section 1.5.

The description of the actions of the cleaning system given above is simplistic, but does provide an idea of the complexity of the processes and the interactions between the different functions. At present, there is no single, all-purpose cleaning system that can be applied to all items of dairy equipment. The major factors affecting the selection of a cleaning system and its performance in a given situation are discussed below.

1.2 THE SURFACES TO BE CLEANED

The nature of the equipment to be cleaned is of importance in three ways. The physical nature of the surface, whether polished, smooth or rough, will determine to a substantial degree, the adhesion of the deposit and the ability of bacteria to survive the cleaning process (Davis 1955). The chemical nature of the surface will impose limitations upon the types of detergents and sanitizers which can be applied. The geometry of the equipment may influence the manner in which the cleaning solutions can be applied.

A number of factors affect the selection of materials used in the construction of dairy utensils and equipment, including availability, strength, thermal conductivity, tainting of dairy products, toxicity to humans, ease of fabrication, cost and also their insolubility in milk, detergents and sanitizers (Clegg 1962). In many countries the materials permitted in dairy equipment are clearly defined (e.g. IAMFES 1973; Standards Association of Australia 1966).

The abrasion which a surface receives markedly affects its cleanability (Ridenour & Armbruster 1953). Wildbrett (1969) related the hardness of the various materials to development of abraded unhygienic surfaces. As the abrasion of a surface increased, more mechanical energy was required for efficient cleaning, which had a further deleterious effect on the surface condition. The wide range of hardness values (HV 0.1 kp/mm^2) of some common dairy materials is illustrated in the following list:

glass - 680 - 725;

18-8 stainless steel - 182 - 192;

half-hard aluminium - 30 - 33;

hard polyvinylchloride - 13;

polypropylene - 8;

linear polyethylene - 4.5;

The relationship between profiles of surface texture and hygienic performance of surfaces has been discussed by Quest (1968).

1.2.1 METALS

The composition and finish of a metal product-contact surface greatly effects its performance in dairy equipment. Pflug et al. (1961) demonstrated in laboratory research that the nature of the surface influenced the rate of soil deposition, but had little or no effect upon soil removal. These findings were confirmed in industry studies (Kauffman et al. 1960a, b, c; Moore et al. 1951). The colour of a surface had no influence on the survival of bacteria on the surface (Gundermann & Schmehe 1970).

Many metals would be satisfactory as product-contact surfaces when newly manufactured with smooth surfaces. However, as the surface of most metals becomes rough and therefore unhygienic at an unsatisfactory rate, the number of metals used in the dairy industry is quite small.

The most important aspect of a metal is its resistance to corrosion. The metal may corrode through the direct chemical effect of detergents, sanitisers or acidic dairy products (e.g. whey, sour cream, fresh cheese curd). Another cause is the electro-chemical action of a pair of dissimilar metals within one item of equipment (e.g. tin and copper - Whittlestone 1961a). The corrosion of a product-contact surface has very important implications because the economic life of the equipment is severely reduced. Also, the corroded surface provides a protected site in which milk residues may lodge and

support the growth of contaminating bacteria (Barnhart et al. 1970a; Daufin et al. 1971; Maxcy 1969b). Cowan (1971) has described the major causes of crevice-corrosion, pitting-corrosion, stress-corrosion, cracking and fatigue-cracking in the dairy situation. Many methods for assessing corrosion have been described (e.g. Mohr & Wortmann 1953; Whittlestone 1963).

STAINLESS STEEL

Stainless steel has become the metal of choice for the construction of almost all items of dairy equipment because of its durability, smooth surface and corrosion-resistance. The manufacture, metallurgy and finishes of various stainless steels have been reviewed by Werra (1974). Stainless steels are resistant to corrosion by alkaline and weak acidic detergents, chlorine sanitizers and brine (Botham 1962; Clegg 1962). There is a large range of stainless steels available with different compositions and properties, but the types favoured for the food industry are the austenitic group, type numbers 302 or 304 for product-contact surfaces,and 316 for other fabrications. Daufin & Talbot (1971) found that the 18/10 was also satisfactory and a 26% Cr, 1% Mo ferritic type was resistant to corrosion by whey (Daufin et al. 1975b).

There are differing opinions on the influence of the surface finish of stainless steel on its cleanability. Langeveld et al. (1972) found a strong inverse relationship between surface roughness and bacteriological cleanability. Scanning-electron-micrographs showed that an unsatisfactory surface with many sites for bacterial lodgement resulted from cold grinding, polishing with a grit of 100 - 180 or electrolytic grinding (Uusi-Rauva & Kosonen 1974).

Satisfactory finishes were observed with polishing with a grit of 220 or greater, or wax brushing. Scratching had no damaging effect, in terms of bacteriological cleanability. Pflug et al. 1961 found that cleanability was slightly affected by surface finish, this effect resulting from differing soil deposition. No relationship was observed between surface finish and cleanability by others in laboratory studies (Kauffmann et al. 1960c; Masurovsky & Jordan 1958) or under field conditions (Futschick 1958; Hay et al. 1958, 1960; Masurovsky & Jordan 1958). The corrosion resistance of cold-rolled surfaces is superior to mechanically polished surfaces (Daufin et al. 1977).

The best surface characteristics for pipelines are on those which are cold-drawn, welded and electrochemically polished (Antila et al. 1975; Plock 1956). However, variations in surface finish have no significant effect in practice on the cleanability of pipelines (Moore et al. 1951; Olson et al. 1960), milk dispenser cans (Kauffmann et al. 1961), refrigerated farm vats (Kauffmann et al. 1961), nor on pasteurisers and pasteurising vats (Kauffmann et al. 1960b).

ALUMINIUM

Aluminium and its alloys are used for some storage vessels, heat exchangers, road transport tankers, cans and milk bottle caps (Clegg 1962; Daufin et al. 1975). They are relatively soft metals, but are light in weight and can be brought to a smooth finish (Clegg 1962). Aluminium is corroded by alkaline solutions, but this may be prevented by the inclusion of silicates in alkaline detergent formulations (Daufin et al. 1975a). These metals are also corroded by acidic solutions, particularly phosphoric acid, although nitric acid can be

used on many alloys (Clegg & Cousins 1968). No abrasives should ever be used in cleaning aluminium (Archibald 1955; Botham & Bryson 1953; Mrozek 1968; West 1947). Aluminium is not as easily cleaned as stainless steel or glass (Ridenour & Armbruster 1953); the cleanability being affected by the type of alloy employed and the surface finish (Masurovsky & Jordan 1958).

STEEL

Mild steel is used primarily in can and bottle-washing machines as it does not corrode with the alkaline detergents used in this equipment. However, a surface scale may develop owing to the precipitation of mineral salts, which can be removed by acidic detergents containing inhibitors (Clegg 1962).

PLATED METALS

Steel and copper base metals with a tinned coating are still used in some items of the dairy equipment, although there is a strong trend towards replacing them with stainless steel for product-contact surfaces. The tin plating can be removed by corrosion by acidic or alkaline detergents or by mechanical abrasion. Once the base metal is exposed, the corrosion rate is accelerated due to the contact of two dissimilar metals. The deposition of milk residue will also be increased (Whittlestone 1961a), giving a very unhygienic surface and contamination of the milk by the base metal (Hankinson 1975). Dissolved oxygen present in the cleaning solution will promote corrosion of tinned surfaces, but this can be overcome by including sodium sulphite in the detergent formulation (Finley & Foter 1947).

The relatively short life of tin-plated surfaces and the cost of stainless steel alternatives initially promoted the development of

tin-nickel-alloy as a replacement protective material for copper or copper-alloy equipment. This had far greater corrosion-resistance than tin and was satisfactorily cleanable (Whittlestone & Lutz 1963). However, the difficulties of plating some awkwardly-shaped articles, and the decrease in cost of stainless steel prevented the adoption of tin-nickel alloy throughout the industry.

1.2.2 GLASS

Glass is used in two types of dairy equipment; as milk bottles and as permanently-installed milk or cream pipelines of cleaned-in-place (CIP) apparatus on farms and in dairy factories. Heat-resistant borosilicate glass with a smooth finish approaches the ideal rigid dairy material as long as dismantling is unnecessary or infrequent. The glass should contain 12 - 14% boric oxide and have a low thermal coefficient of expansion (between 3.0 and 3.5 ppm per ^{o}C, Nielsen 1975). Its smooth surface and transparency are relatively unaffected by chemicals and heat used for cleaning and sanitizing in the dairy industry, and liability to breakage is not serious in practice (Clegg & Cousins 1968; Nielsen 1975). The major practical factor favouring the use of glass is its satisfactory cleanability, which results from its greater resistance to soiling, and from the more efficient removal of the soil that is deposited (Moore et al. 1951; Pflug et al. 1961; Ridenour & Armbruster 1953). The adsorption of some materials to glass, such as phosphates and sodium ions has been demonstrated (Hensley 1951; Hensley et al. 1949), but does not appear to be a problem under practical conditions.

1.2.3 RUBBERWARE

Rubber and rubber-like materials are used on dairy farms for teat

cup liners, tubing and gaskets and in dairy factories for gaskets and hoses. There is a wide variety of rubber formulations, with a range of performance characteristics which permit the appropriate type to be selected for a particular application (Clegg 1962; Salvatierra et al. 1976). Rubber degrades with use; this takes place by the absorption of fat and other milk constituents, by the deterioration of the surface through mechanical or chemical action, and by loss of dimensional stability. Kiermeier et al. (1968) observed that fat and protein films accumulated on the milk-contact surfaces of teat cup liners; these films supported bacterial growth that had adverse effects on the bacteriological quality of the milk, and the organoleptic properties of cheese manufactured from the milk. The deterioration of rubber can be reduced or retarded by efficient pre-milking washing of the teats with soap and water to remove sebaceous fat (Salvatierra et al. 1976a), by washing twice-a-day (Salvatierra & Murphy 1975), and periodic defatting with sodium hydroxide (Salvatierra 1977).

1.2.4 PLASTICS

The use of both hard and soft plastics in the dairy industry has increased greatly in recent years, although they still comprise only a small proportion of product-contact surfaces. The physical properties of those materials have been well reviewed (Dodson 1969; Poulton 1963). Few of the many plastics available have been found satisfactory for dairy use and standards have been established to permit the evaluation of new materials (IAMFES 1964). New items made from plastics generally have a smooth and often shiny surface. Unfortunately, due to chemical and mechanical treatment, in use they quickly lose this hygienically-desirable property. A deposit often develops on the

surface of the plastic item, components of the deposit migrate into the plastic and constituents of the plastic are then able to migrate into cleaning solutions and milk (Rabich 1975; Wildbrett 1969). The plastic ingredients are not toxic to bacteria (Walter et al. 1958).

Many plastics have been evaluated for dairy purposes (Hays et al. 1958; Leesment 1959; Masurovsky & Jordon 1958; Whittlestone 1961c; Whittlestone & Lutz 1962). A considerable study of PVC tubing was reviewed by Wildbrett (1969, 1973). The migration of monomeric plasticisers from PVC into cleaning solutions varies with the formulation and swelling capacity of the plastic, the composition, pH and temperature of the cleaning solution, and contact-time (Wildbrett et al. 1969 ; Wildbrett et al. 1971). These plasticisers are taken up by milk even more efficiently than by cleaning solutions (Wildbrett et al. 1970). Both fat and water are incorporated into the plastic with the result that the plastic loses its flexibility, cracks and thus provides sites for milk soil accumulation and bacterial development (Kiermeier et al. 1968, 1969; Wildbrett et al. 1971). Contamination of milk by these organisms can reduce the bacteriological quality of the milk and impair the organoleptic properties of cheese manufactured from that milk (Kiermeier et al. 1968). Wildbrett (1973) recommended that if plastics materials are used they should be cleaned with a detergent with a pH below 9 and at temperatures not exceeding 50°C. Polystyrene tubing has been used for transporting milk from alpine farms to collection points, with unsatisfactory results in early work done in Germany (Kiermeier & Schattenfroh 1959), but without difficulty in later work in Switzerland (Ritter and Stettler, 1975).

1.3 THE SOIL

1.3.1: THE SOILING PROCESS

A soil has been defined as "matter out of place" (Jennings 1965). The process of soiling is a spontaneous reaction which results in a decrease in the free energy of the system. To reverse the process and clean the surface, energy must be supplied to the system in physical or chemical form. The forces of both attraction and repulsion which act on a soil particle have been reviewed (Jennings 1965; Schwartz & Perry 1949). Derjagvim (1940) and Verway and Overbeck (1948) developed theories deriving the magnitude of the attractive and repulsive forces acting on a discrete particle. Several workers have successfully applied these theories in efforts to account for the forces required to deposit or remove a particle from a surface (Durham 1961; Kling & Lange 1955, 1960; Lange 1957).

Jennings (1965) reviewed many experiments on the aging of soils and reported changes in the proportions of "fast" and "slowly removed" components of a soil with increasing spoilage. Hamilton (1967) examined the potential function derived from the interaction of two colloidal particles and found two potential energy minima as a function of distance between the two interacting particles and concluded that those minima could correspond to the two soil species.

The characteristics of a substrate which can influence its soiling characteristics include its surface finish, porosity, hardness, wettability by a liquid soil component,and chemical reactivity with the soil. The soil characteristics which influence the soiling process include particle size, viscosity, surface tension, mutual solubility of soil components,and chemical reactivity with the substrate (Jennings 1965). The degree of attraction between the deposited soil

and the substrate is related to the polarity of both (Ginn et al. 1961).

1.3.2: COLD MILK AS A SOIL

The effort put into elucidating the chemical and biochemical nature of milk components in recent years has provided information which may be used to explain the behaviour of milk as a soil. Recent reviews on the structure of milk proteins (Bloomfield & Morr 1973; Cheeseman 1975; Lyster 1972; McKenzie 1971; Rose 1969; Slattery 1976), the milk fat globule membrane (Prentice, 1969) and phospholipids (Ansell et al. 1973) have provided valuable background information for this purpose. The soiling properties of milk and milk constituents are enhanced by surface activity. The lower surface tension of milk compared to water results from the surface-active monoglycerides, diglycerides, ceramide hexosides, phospholipids, and casein (Igarashi & Sai to 1972; Morrison 1968).

The composition of a typical cold milk soil is lactose - 38.1%, fat - 29.9%, protein - 26.6% and ash 5.3% (Harper 1972). Pflug et al. (1961) studied the soiling of whole milk and found that the rate of initial soiling varied with the nature of the substrate being soiled. At the completion of the initial soiling a second stage occurred, the rate of which was independent of the surface. Two mechanisms of milk fat deposition have been described; turbulent flow causing fat globule agglomeration, which was attributed to removal of, or damage to, the fat globule membrane, and the formation of an ionically bound insoluble complex between acidic phospholipids and β-lactoglobulin, in the presence of low concentrations of calcium ions (Nakanishi & Kaya 1971a, b). This complex formation could account for the deposition of milk soil when the Ca^{2+} content of a milk residue is

lowered by rinsing (Newlander & Atherton 1964).

Multimolecular protein films which are compressible and non-elastic are formed on the surface of reconstituted solutions of whole milk (Leali et al. 1963). Individual milk proteins vary greatly in their ability to form films with marked differences in both film thickness and film structure (Musselwhite & Palmer 1969). The relationship between pressure-area and pressure-concentration curves of spread and adsorbed films of milk proteins has been studied by Mitchell et al. (1969, 1970). The configurations of spread and adsorbed films are similar (Irons et al. 1970),and Boyd et al. (1973) found that the surface viscosity of spread films varied with both the type of milk protein and the structure of the individual molecules. The conformational characteristics of proteins in these films were found to alter with film formation and surface desaturation (Loeb 1969). Goryaev and Marlin (1970) attributed protein deposition to polarity effects, as they were able to prevent protein deposition during electrodialytic treatment of milk by periodic changes in polarity. Nisbet (1974) found that casein adsorbed to stainless steel surfaces as micelles in a two-stage process - a strong adsorption of casein to stainless steel in a monolayer of micelles,followed by weaker adsorption of casein micelles to the primary layer of micelles. Pre-treatment of stainless steel substrates with chlorinated solutions resulted in deposition of a "chloroprotein" soil, although the exact chemical nature of this soil has not been elucidated (Jensen 1970).

When a surface is repeatedly soiled with milk and washed with detergent, a residual film known as "milkstone" may develop. The chemical composition, physical consistency and appearance of milkstone varies greatly, depending on the nature of soil deposition and accumul-

ation. Some typical analyses show that it contains virtually no lactose, 3.6% - 17.7% fat, 4.1 - 43.8% protein and 42.3 to 67.3% ash (Harper 1972). Roderig et al. (1956) described four classes of "milkstone": soft grey and porous; brown and compact; soft thick brown; and a thin yellow, hard and compact type. Twomey (1966) described three major types which are in good agreement with the above classes. Lavicky and Maxcy (1971) showed that the requirements for yellow film formation were: inadequate cleaning,which resulted in a pre-soil of phospholipids, growth of a high bacterial population of Pseudomonas sp., and available water.

1.4 CONTROL OF DEPOSIT ACCUMULATION BY THE CLEANING SYSTEM

The primary function of a cleaning system is the "removal of food residues that can serve as nutrients for any bacteria remaining on the surface" (Swartling 1959; see Section 1.1). All of the components of a system, including detergents, sanitizers and rinses may contribute to this removal process, but the most important role will be played by the detergent. This section discusses the activity of these solutions in controlling the accumulation of a food soil.

1.4.1: SOIL REMOVAL BY THE DETERGENT

Detergency has been defined as "any procedure for removing liquid and/or solid dirt from the surface of a solid by means of a liquid" (Moilliet et al. 1961). Many books and reviews have been published on this subject. General discussions of the soil removal mechanisms are available (Durham 1961; Moilliet et al. 1961; Schwartz et al. 1958). A particularly valuable review of hard-surface cleaning in the food industry was presented by Jennings (1965) and a more recent review by Kulkarni et al. (1974).

SOIL REMOVAL MECHANISMS

There are three important steps in soil removal: separation of the soil from the substrate, dispersion in the deterging medium and prevention of redeposition on the substrate (Jennings 1965). Most detergents contain surface-active materials which react with the soil and substrate in the following way (Jennings 1965; McBain 1942):

$$\text{Soil} + \text{substrate} + \text{detergent} \longrightarrow \text{soil-detergent} + \text{substrate-detergent}$$

The adsorption of a surface-active material to the soil at the soil-

solution interface reduces the free energy of the soil particle. This results from a reduction of the surface tension at the solid-liquid interface which,if reduced to zero,would permit the soil to dissolve in the detergent. A surface-active material may also penetrate the interstices of particulate soils and disintegrate them.

Once the soil is in the detergent solution it must be dispersed, which may involve disintegration of the soil into smaller particles and transport away from the cleaned surface. This disintegration requires an input of energy which may be physical, but is usually supplied chemically by components of the detergent. In the absence of this energy the soil will again aggregate, such aggregates being far more difficult to maintain in solution (Jennings 1965).

When the soil has been dispersed throughout the detergent solution, the soil particles must be stabilized to prevent their redeposition while the solution is first drained,and then rinsed away (Jennings 1965).

To be effective most detergents require the following activities, to some degree (Jennings 1965):

1. Water conditioning - components of the detergent must inactivate calcium, magnesium and other ions in water which may inhibit the soil removal efficiency of the detergent;
2. Wetting - reduction of interfacial tension to permit rapid wetting, enabling intimate contact between the detergent and soil;
3. Dissolving - mainly a property of the solvent (water);
4. Solubilization - the spontaneous passage of molecules of a water-insoluble substance into a dilute solution of a surface-active agent to form a thermodynamically-stable solution;
5. Emulsification - suspension of oils and fats in an emulsion of

tiny droplets distributed throughout the solvent phase;

6. Peptization - degradation of macromolecules into smaller particles;
7. Deflocculation - disintegration of flocculated aggregates;
8. Rinsing - detergent-soil residues must be readily removed by rinsing;
9. Germicidal activity - sometimes required;
10. Solubility - readily dissolved to prepare use solutions;

There are also some attributes of the detergent which must be minimised. These include:

1. Corrosion - not corrosive to substrates involved;
2. Hygroscopicity - non-caking if powdered concentrate;
3. Cost - economical to use in terms of purchase price, application costs and lack of undesirable side-effects on life of equipment.
4. Lack of toxicological or ecological side-effects.

COMPONENTS OF DETERGENTS

Dairy detergents include surface-active agents, alkalis or acids and builders. The compounds and their characteristics have been thoroughly discussed in the reviews mentioned above and also by Wildbrett (1976).

Surface-active agents (surfactants)

These materials possess, in one molecular or ionic entity, hydrophilic (water-loving) and hydrophobic (water-fearing) structures. The surface-activity of the material is dependent on the balance between these two structures (HLB, hydrophil-lipophil balance; Griffin 1954). The structure must be partly, but not completely soluble in water, and also partly soluble in oil. The associative tendency between water molecules forces the hydrophobic groups from the solution (Durham 1961).

Consequently, the ions or molecules of surfactants are located largely at solvent boundaries or interfaces.

There are three classes of surfactants (Jennings 1965):

1. Anionics - which dissociate in solution to give a negatively-charged surface-active ion and a small inactive cation. These include soaps, amine soaps, sulfated alcohols, sulfated hydrocarbons, alkyl-aryl polyether sulfates, sulfonated amides and alkyl-aryl sulfonates;
2. Cationics - which dissociate in solution to yield a positively-charged surface active ion and a small negative ion. The quaternary ammonium compounds (QAC's) are included in this class.
3. Non-ionics - which remain undissociated in aqueous solution. These include the polyethanoxyethers, ethylene oxide-fatty acid condensates and amine-fatty acid condensates.

The major development in the chemistry of surfactants for use in dairy cleaning has resulted from many authorities requiring the use of more highly biodegradable types. In many situations the substitution of "softer" surfactants has been relatively easily achieved, but with some classes of material, (e.g. the low-foaming non-ionics) equivalent materials of satisfactory performance have not been available.

The extensive work over the last 25 years on the interactions between surfactants and proteins has been reviewed by Steinhardt and Reynolds (1969) and Breuer and Robb (1972). Although the forces acting between proteins and surfactants are understood in general terms, there is currently insufficient information to predict the nature of complexes which will result from a particular interaction. Cheeseman and co-workers (Cheeseman & Jeffcoat 1970; Cheeseman & Knight 1970) observed that the addition of surfactant to a solution of milk casein

resulted in detergent-protein binding which led,first to the dissociation of casein aggregates,and then to further interaction within the casein molecules. The bonds formed by α_s and β-caseins, but not κ-caseins, occurred in parts of the protein molecule containing tyrosine and tryptophan. The ratios of detergent to casein within the complex varied greatly with the type of protein. Green (1971) confirmed the mode of action of the surfactant, which caused disruption of protein aggregates and subsequent formation of complexes with the monomers, which in turn resisted the addition of ca^{2+}. Wide variations were observed, however, in the ability of different surfactants to form protein-surfactant complexes. Hargraves (1972) studied the binding of surfactants to β-lactoglobulin and proposed both general binding on surface sites of the protein,and specific binding in the interior of the protein. The observed alterations in the surface properties of both spread and adsorbed protein films,by the addition of a surfactant,were explained by variations in molecular packing and the influence of surfactants on the protein binding forces (Pearson & Alexander 1968). The only study of the removal of milk protein films from hard surfaces is that of Nisbet and Langdon (1977), who found alkaline anionic detergents to be more efficient than both an acidic anionic and an acidic QAC detergent.

The interactions between surfactants and lipids have been reviewed by Jennings (1965). The two chemically-identical species of lipid that were observed within a soil on a hard substrate were simultaneously and independently removed by two reactions which followed first order kinetics. Hamilton (1967) ascribed the soil removal mechanism to the Narangoni effect, which is due to surface motion created by local variations in surface tension. This is a one-step process where

kinetics are governed by the dissolution of the soil at the soil/air/water boundaries.

Alkalis and Acids

Many acids and alkalis are used in dairy detergents. The compounds used, and their behaviour, are presented in the discussion of classes of dairy detergents, later in this section.

Builders

Both organic and inorganic builders are widely used in dairy detergent formulations. Some of the common builders used in alkaline detergent formulations are carboxymethyl-cellulose, polyphosphates and ethylene diaminetetra-acetic acid (EDTA). The property of major interest with the phosphates is their ability to form soluble complexes with calcium and magnesium hard-water salts. They have the added advantages of disperison, deflocculation, peptizing, and supplying reserve alkalinity. With the organic sequestering agents (e.g. EDTA) the metal from the hard water is bound into an inner ring structure to form a chelate (Jennings 1965).

FACTORS INFLUENCING THE REMOVAL OF SOIL BY THE DETERGENT

Water Quality

The primary solvent for all dairy cleaning solutions is water. The chemical and biological composition, boiling point, surface tension, kinematic viscosity, electrical conductivity and pH are all important properties of the water used for rinse, detergent and sanitizer solutions. The main salts of interest are those of calcium and magnesium which, in the presence of heat or alkali, can react with the HCO_3^- ion to form insoluble carbonates. These precipitates are particularly

difficult soils to remove from surfaces. Iron and manganese salts can also be a problem in specific circumstances (Watrous 1975; Wildbrett 1976).

Contact-time Between Detergent and Soil

Jennings (1965) emphasised that time itself made no contribution to the cleaning process. It is the length of time for which the soiled substrate is exposed to a particular detergent concentration, a particular solution temperature, or subjected to a given mechanical force that is important. Bacon and Smith (1948) investigated the relationship between time of cleaning, detergent concentration and cleaning efficiency. They concluded that the relationship between soil removal time (t), concentration (C) and mechanical force of washing (F) may be described by the following relationship:

$$\text{Soil Removal } (S) = K\,(CFT)^{n}$$

where n represents the slope of the linear portion $\frac{d \log S}{d \log T}$ and K is a constant (Durham 1961). As the concentration of the detergent is reduced and/or the force is decreased, the time must be increased to achieve the same degree of soil removal. Similarly, shorter times may be used if the detergent concentration and/or the mechanical force is increased. The longer the solution is in contact with the deposited soil, the greater the amount of soil that is removed. This reaches a limit, because the final traces of soil are probably never removed and a state is reached where soil redeposition occurs as rapidly as removal (Jennings 1965).

Detergent Concentration

There are two kinds of observation on the effect of concentration on the cleaning efficiency and cleaning attributes of detergents. In

one relationship there is an initial fall and then an increase in detergency with increasing detergent concentration (Palmer & Rideal 1939; Tachibana et al. 1960). In the other, increasing the concentration of the detergent increases the detergency to a limit, and higher concentrations neither increase nor decrease detergency (Harris & Satanek 1961a, b; Jones 1961; Kling & Lange 1955). This is consistent with the idea that a detergent functions by reducing the soil binding forces, but has no influence above a concentration representing some type of saturation effect. These studies all used as detergents either surface-active agents or materials believed to function largely by adsorption and/or displacement. It does not necessarily follow that such a saturation effect is involved with detergent materials such as sodium hydroxide or strong acid cleaners (Jennings 1965). Jennings (1959) examined the removal of milk films from stainless steel by sodium hydroxide and found that a kinetic approach was appropriate and that the reaction was first order with respect to the concentration of hydroxide ion.

Detergent Temperature

There is considerable difference of opinion in the literature regarding the effect of temperature on detergency processes. This is reflected in the wide range of temperatures recommended for practical industry cleaning (e.g. Calbert 1958; Smith 1957). Jennings (1965) ascribed these differences to the use of ill-defined systems, unsatisfactory detergent evaluation procedures and the type of detergents being used. When cleaning an oily soil there appeared to be a small but noticeable increase in cleaning performance with increasing temperature (Bacon & Smith 1948). However, when more complex soils,

particularly those containing protein, were used, there appeared to be an optimum temperature. Schlussler (1970) obtained an optimum cleaning temperature of 85°C for cacao, condensed milk and buttermilk, and 65°C for milk soil. This latter temperature is in good agreement with the results of Polowniak et al. (1960), who described the change in cleaning efficiency with temperature by a quadratic relationship.

The existence of the two species of soil, previously discussed, can make interpretation of experimental data difficult. Bourne (1962) demonstrated that the removal of the more easily-removed species had a negative temperature coefficient. Thus, the net temperature effect on cleaning efficiency would depend upon the relative proportions of each species within a soil. This is related to the age of the soil. Consequently, old soils tend to require higher cleaning temperatures.

The type of cleaning agent used also greatly influences the effect of a temperature change on cleaning efficiency. Surface-active detergents in the micellar form remove soil more effectively and the critical micelle concentration and solubilizing power of the detergent are affected by solution temperature. Certain detergents are heat-labile, and heat, in effect, reduces the detergent concentration. Because soil removal is affected by the detergent concentration, it becomes difficult to divorce the two effects and estimate the effect of solution temperature alone on soil removal (Jennings 1965).

The effect of temperature on simpler detergents, such as water or sodium hydroxide, is relatively uncomplicated. The active material in the latter case is hydroxide ion and the amount of hydroxide ion available is, for all practical purposes, independent of the temperature of the solution (Jennings 1965). Jennings (1959) found that the

rate of removal of cooked-on milk films by solutions of sodium hydroxode increased by 60% for every 10°C rise in temperature (i.e. a Q_{10} of 1.6) within the temperature range of 46°C to 82°C and that this relationship was quite linear with no indication of an upper limit having been reached.

Solution Turbulence

There are two viewpoints on the effect of solution turbulence on detergency when the soiled substrate remains within a continuous detergent solution phase. Bacon and Smith (1948) described the relationship, discussed above, between cleaning efficiency and detergent concentration, mechanical effort, and detergent solution contact-time. However, this relationship implied that all soil is removed eventually, which is contrary to experience (Durham 1961). Schlussler (1970) described a three-part relationship for the cleaning of a milk soil: a threshold level of mechanical effort below which no increase in cleaning efficiency over an unagitated solution was achieved, a phase in which cleaning efficiency improved with increasing mechanical effort, and an upper level of mechanical effort over which no further increase in cleaning efficiency was achieved. The latter two phases of this relationship were observed by Dickinson and Palmer (1951), who noted that the curve of detergency versus mechanical action in the washing of wool reached a plateau, and further increases in energy input did not cause the removal of more soil. Durham (1961) stated that agitation of the detergent solution played an important part in the stability of flocculated systems, as aggregated particles did not disperse spontaneously and the degree of dispersion was dependent upon the time and energy of agitation. After agitation ceased, the

suspension was never stable, and sooner or later the particles aggregated and settled out.

Bourne and Jennings (1961) investigated the effect of turbulence in a discontinuous detergent solution phase and observed two types of mechanical effect on the detergent process. Firstly, a time-dependent process occurred within a continuous solution phase which was related to the phenomena detailed above. Secondly, a time-independent affect occurred,which was related to the advancing and receding air-detergent solution interfaces which passed over the soiled substrate. They named this the "Dupre" effect and this was the major influence upon the level of detergency achieved.

Redeposition of Soil

Redeposition is the readsorption of soil which had already been removed by the cleaning process (Anderson et al. 1959). This may occur if the detergent energy is insufficient to maintain the soil in its dispersed state,and has been observed when a spent detergent solution is passed over clean surfaces (Kulkarni et al. 1975). Hydrophilic colloids such as carboxymethylcellulose can act as anti-redeposition agents. Jennings (1965) postulated that a state may be attained where soil redeposition may occur as rapidly as soil removal, preventing complete cleaning.

CLASSES OF DAIRY DETERGENTS

Dairy detergents have been classified by many systems. The British Standards Institution (1964) has classified by function and defines the following groups.

1. Detergents and detergent-sterilizers for circulation cleaning in the farm industry;

2. Detergents and detergent-sterilizers for hand washing processes, both in farm and processing dairies;
3. Detergents for immersion cleaning of 'direct-to-can' milking machinery;
4. Detergents for cleaning-in-place (CIP) pipelines and milk storage tanks in processing dairies;
5. Detergents to clean equipment which is used for milk heat treatment (e.g. pasteurising and ultra-high temperature (UHT) plants, evaporators);
6. Powdered and granular detergents for bottle-washing in hard and soft water; and
7. Liquid bottle-washing detergents for bulk delivery and storage, which may be used in conjunction with apparatus automatically controlling the detergent concentration.

Conversely, the Standards Association of Australia defined only three major classes of dairy detergents as:

1. Heavy-duty alkaline detergents for 'in-place' cleaning in dairy factories (Standards Association of Australia 1966);
2. Acidic dairy detergents (Standards Association of Australia 1973); and
3. General purpose dairy detergents (Standards Association of Australia 1975).

This latter classification will be used in the following description of detergents.

Heavy-duty Alkaline Detergents

The major components of heavy-duty alkaline detergents include sodium hydroxide, potassium hydroxide, sodium metasilicate, sodium

orthosilicate, sodium sesquisilicate and some amines. The comparative value of these various components in heavy duty alkaline detergents has been tabulated by Somers (1949). In general, the dissolving power of these materials is relatively high and they are reasonable deflocculating agents. They differ in wetting and rinsing properties, and their water-conditioning performance varies from non-existent to only fair. They are effective cleaners for removing most organic deposits, including fats, oils and proteinaceous materials. Strong alkalis have poor rinsing properties, eye-splash is a severe hazard to the operator and they are corrosive toward glass, aluminium, zinc, tin and most paints. Sodium hydroxide is strongly hygroscopic. In the presence of calcium and magnesium the hard-water reactions will produce cloudy insoluble precipitates that may deposit on equipment. Sequestering or chelating agents are used to prevent these reactions (Jennings 1965). Swartling (1950) stated that highly alkaline detergents contain caustic soda as the chief active constituent (70-90%), metasilicate, triphosphate or polyphosphates constitute the major portion of the remainder, together with small amounts of wetting agents. Prepared cleaning solutions usually contain 0.2-1.0% of the concentrate.

General-purpose Detergents

General-purpose dairy detergents are used for light duty cleaning in factories and for cleaning dairy farm equipment. This class includes products which are suited for manual washing procedures and also some to which skin should not be exposed for an extended period. They are often described as "mild alkali" detergents as their common constituents include sodium carbonate, trisodium orthosphosphate, tetrasodium

pyrophosphate and sodium tripolyphosphate (Jennings 1965). Enzyme-containing detergents have not yet been adopted in the dairy industry.

Polyakov et al. (1964) examined many formulations for milking machine cleaning and found a mixture of soda ash, surfactant and anti-corrosive agent to be satisfactory. Twomey (1965) described the general formula given below as being satisfactory:

25% sodium silicate (penta hydrate),

61% sodium carbonate,

10% sodium hexametaphosphate,

2% sodium dodecyl benzene sulphonate,

2% octyl phenol ethylene oxide condensate.

The substitution of either sodium gluconate and disodium EDTA for some part of the sodium carbonate improved detergency, but the latter increased corrosion of tinned surfaces. Watrous (1975) presented formulations for manual and circulation cleaning using either soft and hard waters. These contained varying proportions of sodium carbonate, anhydrous sodium metasilicate, sodium tripolyphosphate and nonionic surfactant.

A number of general-purpose detergent-sanitizers have been prepared by adding sanitizer reagents to detergents. Chlorine-bearing compounds provide sanitizing activity and also enhance detergent performance. Studies on the use of chlorine-supplemented detergent solutions have been reported by many workers (Clegg & Cousins 1970; Kaufmann & Tracey 1959; Lewandowski 1959; MacGregor et al. 1954; Merrill et al. 1961), all of whom found improved cleaning of stainless steel when chlorine compounds were used. The mechanism of these improvements in detergents has been the subject of various hypotheses. Wright et al. (1967) attributed the improved solubility of milk protein

to increased active alkalinity, rather than to the effect of available chlorine. However, Jensen (1970) found that raw milk films were removed effectively from stainless steel plates washed in non-chlorinated alkaline solutions, but film build-up occurred on plates washed in chlorinated alkaline solutions limited to 25 or 50 mg Av. Cl/l. When the concentration of chlorine was increased to 75 mg Av. Cl/l the plates were completely cleaned. He attributed the improved cleaning to the activity of chloride ions, finding no difference in the chloroprotein soil which could be related to changes in active alkalinity. Clegg and Cousins (1970) observed an interaction between the added chlorine and the type of detergent upon cleaning efficiency.

Products containing QAC's which could perform as detergent-sanitizers have been recommended on many occasions. The QAC surfactant is usually formulated with inorganic alkalis or nonionic surfactants (Dahlberg et al. 1950). Whittlestone and Murnane (1962) described the formulation of a product for between-milking soak cleaning of milking machines. This liquid concentrate contained foam stabilizers for foam maintenance in the soak situation, and its formulation was:

20% QAC (cetyl trimethyl ammonium bromide),

1% cetyl alcohol,

0.009% foam stabilizer,

78.991% water.

This product was not used commercially because of deficiencies in sanitizer performance.

Acidic Detergents

Acidic detergents are used for both general-purpose cleaning (cold milk soil) and removing accumulated inorganic soils. Nitric,

citric and sulphamic acids have all been used in the acidified-boiling-water system for cleaning milking equipment (Clough et al. 1965; Palmer & Cowhig 1968). Acidic detergents may also include other acids like phosphoric, or hydroxyacetic, with some surfactants. The cleaning abilities of a range of acids have been compared under dairy conditions by Anderson et al. (1972b) and Haslam and Turner (1960). Many acids pose problems in terms of corrosion of equipment surfaces and dangers to the operators performing the cleaning.

An acidic detergent-sanitizer containing a QAC has been successfully used for milking machines at temperature of 80°C (Heyes & Dunsmore 1978). Some high-acid iodophors are also used as detergent-sanitizers (Twomey 1968-69).

1.4.2: DETERGENCY IN PRACTICE

SOIL REMOVAL BY THE WHOLE CLEANING SYSTEM

The detergent may be the most important component of the system in terms of deposit control, but many other components also make significant contributions.

Rinses

In any system the sequence after soiling may be: post-product rinse, detergent A, rinse after detergent A, detergent B, rinse after detergent B. The post-product rinse plays a very important role in cleaning, by removing the majority of the food residue which is merely lying on the surface, and leaving that which is adsorbed. This greatly reduces the soil load on the detergent and leaves only the adsorbed soil, which requires the detergent for removal (Beattie 1958a, b). The rinse between two detergents (rinse after detergent A in the system described above) is used to remove as much of the residues of

the soil and first detergent as possible. This eliminates the possibility of the reagents of the first detergent inactivating the second, and also reduces the soil load on the second detergent. The final rinse (rinse after detergent B in the system described above) is used primarily to remove the residues of detergent B and soil from the surfaces, and if hot, may be also used as a sanitizing agent.

Attempts have been made to improve the performance of the rinses by altering the temperature, or by adding reagents ("rinse additives"). In milking machines, the rinsing efficiency was improved by raising the temperature from 15.5 to 46°C and by adding a nonionic surfactant, but not an anionic surfactant (Calder & Twomey 1966; Clegg & Cousins 1970; Patel & Jordan 1964). The re-use, as a post-product rinse, of a detergent retained from the previous cleaning was unsatisfactory (Clegg & Cousins 1970). The addition of an acid to the final rinse reduced the soil by removing some of the minerals, and could also prevent the deposition of hard water salts from the final rinse solution (Clegg & Cousins 1970).

Other Detergents and Sanitizers

The fact that many soils contain both alkali-soluble (organic) and acid-soluble (inorganic) components has led to the complementary use of both acidic and alkaline cleaning solutions. Often the main detergent is applied routinely and the complementary detergent periodically. Sometimes the complementing detergent is applied as a second detergent at every washing, or in the form of a detergent-sanitizer (e.g. iodophor in milking machine cleaning - Scott et al. 1962). Sanitizers may also contribute to soil removal themselves (e.g. as discussed above chlorine degrades milk proteins).

SOLUTION APPLICATION METHODS

The removal of a soil requires an input of energy. The equation of Bacon and Smith (1948), described in Section 1.4.1, showed the importance of the contribution kinetic energy can make to detergency. In practice, kinetic energy can be supplied by cleaning solutions when they are applied by flowing through closed pipelines, falling as thin films down open surfaces, or impinging on open surfaces from sprays. Jennings et al. (1957) investigated the effect of solution turbulence (expressed in Reynold's numbers) on the cleaning efficiency of detergent solutions recirculated in a pipeline. No detergency improvement was noted until the Reynolds number attained 25,000, after which detergency improved linearly with increasing solution turbulence. Jennings (1965) later ascribed the effect of turbulence to the Dupre effect, as rising solution turbulence increased the incidence of entrained air, which caused more frequent contact of the soil by air-solution interfaces. The application of pulsating pressure to solutions flowing in a pipeline increased the removal of soil, the improvement depending upon the frequency and amplitude of the pulsations (Surkov et al. 1974).

PERIODIC CLEANING

No cleaning system is completely effective in removing the soil. Consequently, over a period of time, a soil will build up which is resistant to the routine cleaning system. This soil provides a site for bacterial growth which is protected from the action of chemical sanitizers (Clegg & Cousins 1968; Major 1962b; Mrozek 1974). Consequently, a different cleaning system, which is more effective at removing the accumulated soil, is applied periodically.

On the metal parts of milking machines the residue of the usual

alkaline detergent is an alkali-insoluble (acid-soluble) "milkstone" which is primarily mineral and protein. It has been recommended that this deposit be removed by acidic detergent "de-scalers" whenever necessary (Calbert 1958), monthly (McCulloch 1965), or weekly (Whittlestone 1954). More recently, acidic treatments have been incorporated into the routine cleaning system applied at every use. Scott et al. (1962) used the acidic-detergent nature of the iodophor sanitizer for this purpose, and normal acidic detergents, or acidic-anionic sanitizers could also be used (Clegg & Cousins 1968). Roderig et al. (1956) found that the composition of the milkstone on metal surfaces varied greatly, which altered the cleaning requirements. Rubberware is a slightly different problem, as fat is the most adherent soil on this material and should be removed by a periodic treatment of caustic soda (Salvatierra, 1977).

CORROSION

The corrosion of the surfaces of dairy equipment by cleaning solutions creates sites where a soil will adhere. Such a soil will protect occluded bacteria from detergent solutions and permit them to grow and contaminate the product (Maxcy 1969). The corrosion of metallic surfaces during the cleaning process has been well reviewed (International Dairy Federation 1971; Jennings 1965; Wildbrett 1967) and also discussed in Section 1.2. The constraints placed upon the selection of detergent formulations by the nature of the surface of the equipment to be cleaned has often been demonstrated, most recently by Szego and Keresztessy (1965) on a range of Hungarian dairy detergents on a variety of metals. Lindquist and Magnusson (1966) found that a general-purpose detergent which contained silicate corroded

aluminium below 80°C and Daufin et al. (1970) reported that every one of a range of detergents, detergent-sanitizers and sanitizers corroded the aluminium alloy "Almasilium" to an unsatisfactory degree. The amount of corrosion of stainless steel by chlorinated-alkaline detergents was small, being related to the concentration of hypochlorite in the formulation (Merrill & Jensen 1961).

Similar studies have also been performed with acidic detergent materials. Following their studies on the corrosion of tin, tin-nickel alloy and stainless steel, Whittlestone and Lutz (1962) recommended the use of an iodophor containing 9% in preference to 16% phosphoric acid. Fluckiger (1970) reported on the corrosion of galvanised iron and the alloy "Anticorodal" after exposure to a range of acidic reagents and proprietary acidic detergents.

FOAM FORMATION

The theory of foam development, stability and breakdown has been well reviewed (Jennings 1965; Schwartz & Perry 1949; Schwartz et al. 1958). The importance of foam in detergency has been subjected great deal of examination which has resulted in some diverse opinions being expressed. Bikerman (1947), in reviewing the literature, found that the views on the importance of foam in detergency ranged from "none" to "essential" and Jennings (1960) even described a situation in which foam inhibited detergency. Moilliet et al. (1961) attempted to resolve these apparent conflicts by stating that foaming power is related to the presence of ionic aggregates while detergent power is determined by the molecularly dispersed portion of the surface active agent. Such evidence eliminates foaming as a fundamental phenomenon in the detergent process. In practice, high-foaming detergents may

be advantageous when cleaning large-volume equipment, as they reduce the amount of detergent solution required to make contact with all of the equipment surfaces. However, high-foaming detergents may be disadvantageous where the solutions are recirculated through pipelines, as in the milking machine. Where necessary, defoaming agents can be incorporated into the detergent (Gast 1972).

EXHAUSTION OF DETERGENT ACTIVITY

Moilliet et al. (1961) stated that any phenomenon which decreases the active concentration of the detergent during a detergent process will have an unfavourable effect on cleaning performance. There are two major mechanisms of detergent exhaustion, the first of which is the adsorption of the detergent onto the soil and the substrate. Detergent losses through adsorption to the soil which is being removed is inescapable, but the losses through adsorption to the substrate may be avoided or minimised in situations in which:

1. Lowering the substrate-solution interfacial tension is unnecessary for the liquid dirt to be displaced;
2. Removal of oily dirt does not need to be complete; or
3. Adsorption of detergent by solid particles alone is sufficient to detach them from the substrate.

The second mechanism of exhaustion is detergent decomposition or precipitation (Moilliet et al. 1961). This mechanism has been demonstrated by Schlussler (1970) who studied the effect of soil load on detergent solutions and noted that the decomposition products of the soil-detergent reactions will affect the solution and its cleaning action differently to freshly-added soil. The cleaning capacity of a solution will have a different limiting value with every cleaning

agent and every type of soil. The classic case of precipitative exhaustion occurs with lime soaps, resulting from reaction between the surface active agents and hard-water ions, and has the secondary deleterious effect of settling out on the substrate. This may be overcome by the use of "lime-soap dispersing agents" or sequestering agents (Moilliet et al. 1961).

ECONOMISING ON CLEANING COSTS

Detergent Re-use

The re-use of detergent solutions has become an industry practice, without a scientific understanding of the factors involved. Smith and Hedrick (1967) re-used a chlorinated alkaline detergent in a factory situation and achieved a satisfactory standard of hygiene with a reduction in raw material costs, and Reeves and Tilley (1972a, b) re-used both iodophor and chlorinated-alkaline detergents for road transport tanker washing. Price et al. (1976) re-used an alkaline detergent for 12 washings on a milking machine, saving 80% of the detergent costs, 60% of the heating costs and 30% of the water costs.

1.5 USING THE CLEANING SYSTEM TO CONTROL BACTERIAL CONTAMINATION

Bacteria undergo a "contamination cycle" on food equipment surfaces. The cycle has the following components:

1. Deposition - the bacteria may settle on the surface from many sources;
2. Attachment - some of the deposited organisms attach;
3. Depletion - detergents and sanitizers are used to remove and kill as many organisms as possible;
4. Development - some of the organisms that survive the cleaning system may grow if conditions are appropriate;
5. Contamination - the next time the food passes over the surface some of the viable organisms may be detached from the surface and contaminate the food.

The different phases of the contamination cycle will be discussed below.

1.5.1: DEPOSITION OF BACTERIA ON EQUIPMENT SURFACES

The milk which soils a milking machine contains organisms which are produced in the udder and others which are associated with the teat skin. Aseptically drawn milk contains from 10 - 1,000 bacteria/ml in non-diseased animals and up to 10^8/ml where the udder is diseased. The flora is limited by the biochemical conditions within the udder to a few types, mainly micrococci, streptococci (*Streptococcus zymogenes*) and some rods (*Corynebacterium bovis*, and *Brucella lipolyticus*) (Bacic et al. 1967; Foster et al. 1958; Kleter & de Vries 1974).

The milk may be contaminated by diseased udders, from cow housing

and bedding, and from udder washing. The udder disease which most commonly increases the milk bacterial count is mastitis. A mastitic cow may produce milk containing up to 10^8 bacteria/ml, the major organisms being Streptococcus agalactiae, Streptococcus pyogenes and Staphylococcus aureus, E. coli and pseudomonads (Dodd & Neave 1970). The numbers of S. aureus may fluctuate widely from none to 10^8/ml with 24h (Sharpe et al. 1962). Under these conditions these organisms constitute the majority of the total count (Cousins, 1972). The other major pathogenic organisms that may be present in milk are Brucella abortus (brucellosis), Mycobacterium tuberculosis (tuberculosis), Coxiella burnettii (Q fever) and Leptospira pomona (leptospirosis).

During the milking operation the milk may be contaminated from the exterior of the udder by up to 20,000 organisms/ml (Cousins 1972; Johns 1962a). This level of contamination can largely be eliminated by udder washing prior to milking (Cousins 1972; Moore 1955).

The air may be a significant vector for bacteria, permitting them to deposit on the product-contact surfaces of the milking machine. The microbiology of the atmosphere has been exhaustively reviewed by Gregory (1973). Moving air does not normally detach bacterial cells from the surface of a colony, but the mechanical disturbance of dust, clothing, soil etc. can carry contaminated particles of the substratum bearing clumps of bacteria ("rafts") into the air (Bourdillon & Colebrook 1946). Bacteria may also become airborne in splashes from a contaminated solution. The distribution of bacteria in the air is random and can vary greatly in short periods or over small distances. Air borne bacteria may contact a surface through sedimentation, boundary layer exchange, turbulent deposition, rain-washing or electrostatic deposition (Gregory 1973).

The factors affecting the numbers and types of aerial organisms in the air include (Gregory 1973):

1. Rain - decreases the numbers greatly;
2. Circadian periodicity - the numbers vary greatly during the day, the pattern changing with the site;
3. Weather;
4. Season;
5. Biotic factors - there is a transient increase in the aerial contamination of dairies during sweeping, handling feeds, handling stock and more permanent increases where there are dirty floors, walls and ceilings. The flora will be related to the flora of the source (e.g. hay, Baruah 1961; Majewski 1970).

The amount of aerial contamination will depend on the area of the product-contact surface which is exposed to aerial contamination between milkings, and the volume of air drawn into the milking machine by vacuum, through the air admission holes. Data by Benham & Egdell (1970) show this latter mode of milk contamination to be negligible, contributing fewer than 60 bacteria/ml milk. The distribution (%) of micro-organisms was: Gram positive cocci 50 - 70; Gram positive rods 10 - 40; Gram negative rods 10 - 40; aerobic sporeformers 7 - 91 and moulds 4 - 10.

Personnel can contribute to the bacterial population on equipment surfaces by direct contact or by indirect contamination via the air, or via cow's teats. The hands of a dairyman may support a large number of bacteria including pathogens (Lavantia & Singh 1973; Majewski 1967; Moeller et al. 1963; Smythe 1960; Waes 1973). The numbers may be reduced, but not eliminated by hand disinfection (Neave et al. 1962). Milk can also act as a vector for diseases such as typhoid and para-

typhoid fever, dysentery, scarlet fever, septic sore throat, diphtheria and cholera (Foster et al. 1958).

The cleaning solutions applied to food equipment surfaces may be important sources of bacterial contamination. Those solutions which contain detergents, sanitizers and combined detergent-sanitizers are, to all intents and purposes, sterile and therefore pose no contamination problem. However, the water used for rinsing is often highly contaminated and can contribute significant numbers to equipment surfaces. Where the rinse is applied before a detergent or sanitizer, any contamination will probably be controlled. However, where the rinse water is applied as the last solution before the food - usually to prevent sanitizer residues entering the food - the contamination will remain on the surface until the equipment is used. The fact that cleaning solutions are often recirculated around pipelines many times makes then an ideal vector of bacteria from one part of the equipment to another.

Two other methods of contaminating equipment surfaces are by contaminated cleaning apparatus (e.g. brushes) and insects and rodents. However, these are not usually significant sources in practice.

1.5.2: THE ATTACHMENT OF BACTERIA TO SURFACES

Little research has been conducted into the attachment of bacteria to the product-contact surfaces of food equipment. However, bacteria are attached to surfaces in many micro-environments of practical importance and information from studies of these sites may be applied to the situation in the dairy industry. The micro-environments which have been studied include the surfaces of marine locations, mineral and soil particles, effluent treatment plants, leaves and roots of plants, the mouth, intenstine and skin of man and animals. The attachment of bacteria to surfaces has been well reviewed recently by Marshall (1976).

The site which has received the most study is that of the tooth surface because of its importance in dental health. The subject has been reviewed by Gibbons and van Houte (1975), Newman (1974) and Newman and Poole (1974). The tooth can serve as a useful model for the food equipment surface as it is a hard crystalline surface which has some surfaces well exposed and others which are protected from the external environment. The tooth surface is maintained at a temperature favourable to bacterial growth, is periodically soiled with foods,has its surfaces bathed in a cleansing solution (saliva), and is physically cleaned. Some further information,from bacterial attachment to hard surfaces in the marine environment,is also instructive (Marshall 1976).

Many, if not all,bacterial cells have hydrophilic and hydrophobic ends, in a similar way to molecules of surface-active agents (Marshall & Cruickshank, 1973). This property results in migration to interfaces and, in the case of rods, their orientation perpendicular to the interface,with the hydrophilic end in the aqueous phase and the

hydrophobic end in the non-aqueous phase. It can also result in rosette formation in an aqueous medium by a number of organisms, all with their hydrophobic ends towards the centre, similar to micelle formation by surfactant molecules.

Once on a surface, motile organisms have been observed initially to attach and detach readily, either by a polar flagellum or fascicle of flagella, or along their length. In these states they exhibited violent movement due to the action of flagella or Brownian movement. Some of the bacteria attached along their edges became non-motile (Marshall et al. 1971b; Meadows 1971).

Zobell (1943) observed that the firm attachment of micro-organisms to surfaces required an incubation period of 6 - 12h. Marshall et al. (1971b) described two phases of attachment: a reversible attachment where the organism was held weakly near the surface and still exhibited Brownian motion, and an irreversible attachment where they did not exhibit Brownian motion and were not easily washed off. Sometimes micro-colonies, rather than single cells, also attach (Saxton 1973).

Once attached the organisms' behaviour changes from the non-attached condition. They have a low or negligible rate of cell multiplication and consequently have thickened cell walls, and large amounts of intra-cellular polysaccharide (van Houte & Saxton 1971). In some sites there may be a succession of organisms. For example, Corpe (1974) described a series in a marine situation which commenced with single cells or micro-colonies of rod-shaped bacteria, followed by stalked and filamentous bacteria, which were succeeded by diatoms and protozoa. On the surfaces of teeth, the initial organisms attaching were facultative or aerobic, but these were later replaced by anaerobic

organisms when a deep plaque is formed (Loesche 1975).

Little is known of the precise mechanisms of attachment. In a number of micro-environments an organic layer is adsorbed to the surface first. On teeth this acquired pellicle contains salivary glycoproteins which may competitively inhibit the attachment of bacteria (Gibbons & van Houte 1975; McGaughey et al. 1971). Protein may attach to hard surfaces in the marine situation, and may either facilitate or impair the attachment of bacteria (Fletcher 1976; Meadows 1971).

When an organism is in the reversible state of attachment it is attracted by weak London-van der Waals forces and repelled by the negative electrostatic (double layer) energies of the opposing surfaces. The organism is held at a point of secondary minimum energy, and neither thermal nor kinetic energy is sufficient to overcome the repulsion barrier near the surface (Marshall et al. 1971b). This description was confirmed by Fletcher's (1977) observation that the attachment of bacteria to polystyrene could be fitted by Langmuir-type equations, which described the adsorption of non-biological materials to surfaces.

Two mechanisms of irreversible adsorption have been proposed. The proposal put forward by Marshall et al. (1971b) that this may be achieved by polymer bridging, has been confirmed. A number of bacteria have been shown to synthesise extracellular polysaccharides (EPS) when weakly associated with a surface, providing the irreversible attachment (Clarke et al. 1974; Fletcher & Floodgate 1973; van Houte & Upeslacis 1974; Marshall and Cruickshank 1973). This was facilitated by the presence of a carbohydrate-rich cell coat on the surface of most, if not all cells. The incubation time of 6 - 12h required for irreversible attachment is the time required for the de novo synthesis of the

EPS. The chemical nature of this EPS is variable, the attaching efficiency of some organisms being related to the proportion of $\alpha(1 \rightarrow 3)$-like linkages in the glucans (Johnson et al. 1977; Pearce 1976). This variation in composition is related to the proportion of an enzyme complex operating at any time (Mukasa et al. 1974).

The second mechanism proposed to explain irreversible attachment is that using attachment appendages. Many organisms in the mouth possess a fibrillar coating which mediate their attachment to surfaces (Gibbons & van Houte 1975). Fimbriae, which are small filamentous appendages having no motility function, have also been suggested as having considerable importance in attachment (Newman 1974).

Many organisms are not capable of attaching directly to surfaces. However, they can still fill an important ecological role by attaching to dissimilar organisms which are already adsorbed to a surface. This has been well demonstrated in the oral micro-environment, where several very specific associations have been reported (Gibbons & van Houte 1973; Russell & Coulter 1977).

Many factors affecting the efficiency of bacterial attachment have been studied. The bacterial numbers in suspension have been found to directly affect the numbers attaching, a concentration of $10^3 - 10^4$ bacteria/ml being required before significant attachment occurred. This number varied with the organism used (van Houte & Green 1974). The rate of attachment has been found to increase with temperature, time of exposure of the surface to the organisms (Fletcher 1977), and the pH and electrolyte concentration of the solution (Fletcher & Floodgate 1973). Organisms in the logarithmic growth phase have been found to attach at a greater rate than those in the stationary or death phases (Fletcher 1977). The evidence on the

attachment of dead cells is slightly contradictory. Ultra-violet treatment and low-heat treatment permitted attachment, but formalin or high-heat treatments inhibited attachment (Corpe 1974; Meadows 1971; Pertsovskaya & Zvyaginstev 1971).

The nature of the surface has also been found to indirectly influence the attachment of bacteria. The surface affected the nature of the monolayer of material which adsorbed to it, and this monolayer in turn determined the attachment behaviour of the bacteria. Dexter et al. (1975) reported an influence of substrate wettability on bacterial attachment through this mechanism. Further, in marine environments, the existence,in non-lethal concentrations,of any bacterial repellent markedly reduced the attachment of motile bacteria, and also the formation of slime,which was dependent on their attachment (Chet et al. 1975).

The major influence on the attachment behaviour is the suitability of the organism to the microenvironment. In the mouth there are a number of well defined micro-environments and each has its own characteristic microflora (Gibbons & van Houte 1975). Each site has its characteristic pH, redox potential and nutrient supply, yet Gibbons and van Houte (1975) have demonstrated that the primary determinant of an organisms maintenance at these sites is their attachment performance. For an organism to be maintained in the population where fluid is flowing over the surface it must either multiply at a rate greater than the dilution rate, or attach to surfaces. The multiplication rates are in general insufficient for this purpose, which leaves attachment as the major influence. In the mouth the various salivary glycoproteins which attach to the tooth surfaces in the acquired pellicle are responsible for the binding of specific organisms. Also,

saliva contains immunoglobulins which have also been shown to influence attachment (Gibbons & van Houte, 1975). Thus, the types of bacteria which attach to teeth are controlled to some extent by the nature of the host's salivary secretions, (Gibbons and van Houte, 1975). The implications and applications of these findings for the dairy industry requires further study.

The attachment of bacteria to surfaces has a number of important implications. It permits the organism to colonise a site where fluid flow would otherwise remove it (Gibbons & van Houte 1975). In micro-environments where the level of nutrients in the bulk solution is insufficient for growth, the concentration of the nutrients at interfaces permits growth of organisms at those locations (Corpe, 1974). Furthermore, bacteria killed by most forms of physical and chemical stress in the natural environment are unlikely to attach, which means that the surfaces will be colonised by living rather than by dead organisms (Meadows 1971). Finally, the direct relationship between the ability to attach to some surfaces and pathogenic virulence, means that there will be a selection for pathogenic organisms on surfaces (Gibbons & van Houte 1975).

1.5.3: DEPLETION OF BACTERIA ON SURFACES

The numbers of the bacteria that are attached to surfaces of the equipment will be depleted by the physical and chemical agents in the cleaning system. The effects of these agents on the bacteria are discussed below.

TEMPERATURE

The Temperature of Milking Machine Surfaces

The temperature of the surfaces of milking machines range from less than 0°C to greater than 100°C. Low temperatures occur when equipment is exposed to sub-zero ambient temperatures and high temperatures where the hot detergent or sanitizer solutions are applied. The temperatures of the surface are often not constant, but cycling. Many surfaces will vary diurnally with ambient temperatures. Milking machines cleaned with hot solutions will also have a 12 hourly cycle.

Heat has been applied to milking machines in two forms for sanitizing purposes: steam and near-boiling water. Steam has the advantage of being lethal for most vegetative cells and active against spores. By heating a soil on an equipment surface it can kill bacteria lying within the soil which may not come into contact with, and therefore not be affected by, chemical sanitizers. At any temperature steam is more effective than boiling water because, when condensing on a surface, the latent heat of vaporisation is given to the surface. Steam can be applied either to equipment contained in enclosed, non-pressurised "steam chests", or by jets into enclosed spaces like pipelines. It is normally applied at atmospheric pressure $\sim$ 100°C for 10 minutes. It is non-corrosive to equipment, but lacks persistence

of effect (Clegg 1962; Davis 1956a). The requirement for long cooling periods is not a problem with the 12 hourly cleaning cycle with milking machines. Steam has been applied to dismantled milking machine parts in steam chests, in which case care must be taken with rubberware. Pipeline milking machines can also be treated satisfactorily but, if the milking vacuum level is maintained while steaming, the temperature cannot exceed 82.5°C (Whittlestone, 1961).

Hot water is also a popular method of heat sanitizing dairy equipment, having the same powers of penetration, although it is not as bactericidal as steam. However, it can be a useful treatment where steam facilities are unavailable or steaming is inappropriate. Hot water is usually applied until the exhausted water reaches at least 85°C for at least one minute (Clegg 1962). Hot water has traditionally been recommended as the sanitizing agent in milking machine cleaning systems (Calder & Twomey 1966; Clough et al. 1965). However, in the iodophor/alkali system, where the alkaline detergent is applied hot and an iodophor sanitizer is applied immediately before milking, the post-detergent hot water rinse does not contribute significantly to the bactericidal performance of the system (Hubble et al. 1976; Twomey & Crawley 1969a).

The Effect of Low Temperatures on Bacterial Survival

Low temperatures can adversely affect the viability of bacteria by two mechanisms: cold shock and freeze-thaw damage. Both of these situations may occur on the dairy equipment surface.

Cold shock, which is the sudden chilling of a dilute suspension of bacteria from their normal growth temperature to near 0°C, can cause a great loss of viability (Sherman & Albus 1923). It is

accompanied by the release from the cell of a number of low molecular-weight intracellular solutes including nucleotides, amino acids (Strange & Dark 1962; Strange & Ness 1963; Ring 1965), K^+ (Haest et al. 1972) and low molecular-weight proteins (Smeaton & Elliot 1967). Also, the DNA of cold-shocked organisms contains more single strand breaks than that from unshocked organisms (Sato & Takahashi 1970). The permeability changes arise from a phase transition in the membrane lipids. (Farrell & Rose, 1968; Haest et al. 1972; Leder 1972; Ring 1965). The sensitivity of organisms to cold shock is influenced by a number of factors which are summarised in Table 1.5-1. Recovery from cold shock has sometimes been observed. This can be facilitated by inclusion of specific nutrients or reagents in the medium, including divalent ions or NAD or ATP (Sato & Takahashi 1968, 1969). The practical significance of cold shock in the milking machine environment has not been established.

During freezing and thawing a cell is exposed to changes in temperature, water content and solute concentration. The cell is initially exposed to ice formation in the extracellular medium, while the intracellular water content remains supercooled, often down to $-10^{\circ}C$ (Mitchell & Moyle 1956; Scott 1962). With slow cooling, the supercooled internal water will tend to flow out of the cell because it has a higher vapour pressure than the external ice, and thus freezes externally; but with rapid cooling a cell will be unable to lose water sufficiently quickly to maintain equilibrium, and its water will become supercooled and freeze within the cell. As the formation of ice in a cell or its surroundings removes water, it concentrates both intracellular and extracellular solutes. At some temperature (the eutectic point) the concentration of solute will exceed its solubility

Table 1.5-1: Factors affecting the sensitivity of bacteria to damage by cold shock

Factor	Comments	Reference
Age of culture	Cells in logarithmic more susceptible than those in stationary phase.	Hegarty & Weeks, 1940; Meynell 1958
Composition of growth medium	Complex media more protective than chemically defined media.	Farrell & Rose 1968
Composition of chilling medium	Divalent cations (Mg^{2+}, Ca^{2+}, Mn^{2+}) provide protection.	Strange & Dark 1962
Cell population number	Low numbers more susceptible.	Strange & Dark 1962
Cooling rate and range	Faster chilling causes greater damage.	Leder 1972; Smeaton & Elliot 1972

and the solute will precipitate along with the remaining water as an intimate mixture of ice and solid solute (Hagen, 1971).

When organisms are thawed there is considerable damage to both cell membrane and cell wall. Keith (1913) suggested that the predominant factor in bacterial destruction at low temperatures was the formation of ice crystals, which either compressed or speared the cell from outside, or ruptured them by expansion from within. This suggestion received support by a number of early workers. However, the work of Harrison (1955) showed that, during freezing and thawing, certain species of bacteria are not damaged by the mechanical crushing of ice crystals, but by the action of concentrated electrolytes at temperatures above their eutectic point. The increased permeability of the cell membrane of freeze-thaw damaged cells is shown by the leakage of intracellular solutes into the medium (Sakagami 1959), and the increased penetrability of some compounds into the cell (Strange & Postgate 1964). However, it appeared that this membrane damage was not the primary cause of death, and the exact site of the freeze-thaw damage has yet to be identified (Hagen 1971).

The numerous factors which have been shown to influence the survival of freeze-thaw damage by bacteria have been summarised in Table 1.5-2. Freeze-thaw damage may occur on milking machine surfaces in areas where there are low ambient temperatures, but its practical significance as a bactericidal agent has not been determined.

The Effect of High Temperatures on Bacterial Survival

Thermal energy is always present to some degree in a bacterial cell and the capacity of a cell to insulate itself from changes in the environmental temperature are negligible. Unlike toxic chemical

materials, any heat treatment will always be a quantitative effect which not only affects the cell as a whole, but every molecule and every reaction within it (Brown & Melling 1971).

Many sites within the cell have been associated with thermal death, but logically the most important site would appear to be that biochemical system essential to viability which is also the most heat sensitive. However, Welker (1976) concluded that,on the basis of the available evidence,there is no one site responsible for the heat inactivation of the bacterial cell. The cell wall may act as a poor insulator to protect the underlying membrane from thermal injury, but this does not seem significant in practice. Although the composition of the cytoplasmic membrane of thermophilic organisms differs from other organisms (Wisdom & Welker 1973), it appears that damage of this membrane is not the primary thermal injury (Allwood & Russell, 1970). Although there is no relationship between DNA thermostability and thermophily, the production of single strand breaks in DNA may be the primary event in thermal inactivation (Welker 1976). The ribonucleic acids of thermophilic bacteria are not different from those of mesophiles, but the ribosomal proteins or membrane (DNA attachment site) play an important role in the functional integrity of nucleic acids at elevated temperatures (Welker 1976). Also, the proteins of thermophilic organisms have been shown to possess inherent thermostability, which is dependent upon the amino acid composition and sequence, which permit additional bonds to stabilize the secondary and tertiary structures of those proteins (Welker 1976). Information on the many factors which influence the heat stability of any organism are summarised in Table 1.5-3 .

The kinetics of thermal death have received considerable study

Table 1.5-2: Factors affecting the ability of a bacterium to survive freeze-thaw damage

Factor	Comments	Reference
Type and strain of organism	Some survive most freeze/thaw treatment; some resist immediate consequences of low temperatures but die in subsequent storage; others are susceptible to immediate and long term damage.	Hagen 1971
Population density		Major et al. 1955
Nutritional status	Aerobes' survival facilitated by aeration and inclusion of sodium oleate in medium	Macleod & Calcott 1976
Growth phase	Cells in early lag phase more susceptible than those in stationary phase	Toyokawa & Hollanders 1956
Composition of freezing menstruum	Salts decrease survival, compounds like glycerol & polyvinyl pyrrolidone are cryoprotectants	Macleod & Calcott 1976
Cooling rate to freezing point of suspension	Optimal cooling rates vary with the organism and cooling menstruum	Calcott & Macleod 1975
Cooling rate from freezing of suspension		Mazur 1966
Time held at low temperature		Harrison 1956
Holding temperature	Lower temperatures increase survival	Straka & Stokes 1959
Rate of warming to freezing point		Mazur 1966
Dilution of medium before enumeration		Bretz & Hartsell 1959
Repetition of free-thawing	Multiple freeze-thawing greatly increases damage	Harrison 1955
Repair of freezing damage	Complex media facilitate repair	Macleod & Calcott 1976

Table 1.5-3: Factors affecting the ability of a bacterium to survive high temperatures

Factors	Comments	References
Species and strain variation	Great variation between species and strain; significant changes not achieved through selection.	Morrison & Rettger 1930; Anagnostopouloset al. 1966.
Phase of growth	Cells in stationary phase more heat resistant than lag-phase cells - confounded by incubation conditions.	Strange & Shon 1964 Brown & Melling 1971
Conditions of heating	Resistance to heating increases in absence of H_2O and O_2, the presence of sugars, electrolytes, salts and possibly fats, and at optimal pH.	Brown & Melling 1971
Conditions of recovery from heat damage	Damaged organisms require more complex media; optimal conditions altered	Nelson 1943, 1956
Spore production	Spores are very heat resistant - the degree varying with the organism, numbers present, phosphate and divalent cation content,and pH of medium, cultivation temperature, water activity before and during heating and the composition of suspending medium during heating	Russell 1971

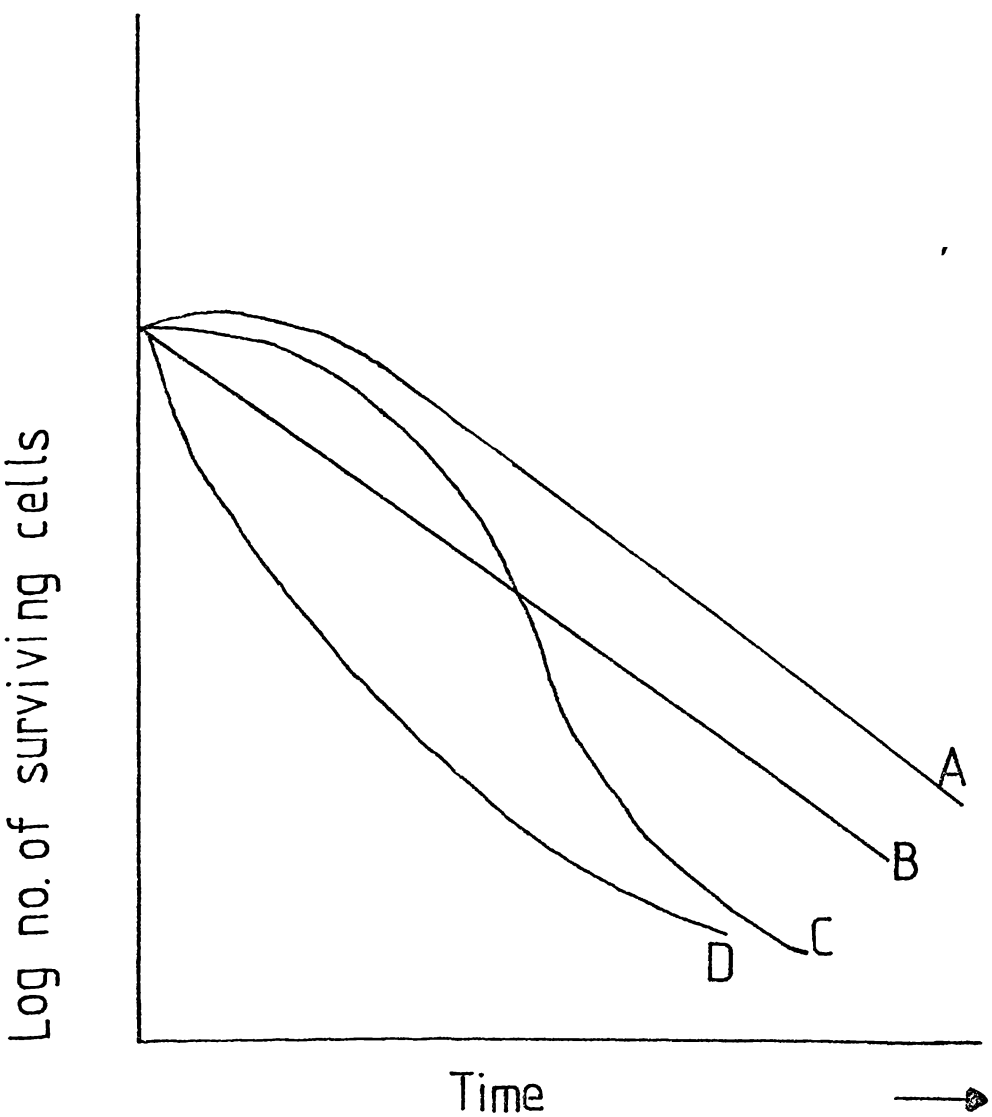
and the early conclusions were that bacterial death was exponentially related to the time of heat application. More recently a number of variations to this relationship have been observed and these are presented diagrammatically in Figure 1.5-1. The logarithmic death rate (B) was explained by a unimolecular reaction model in which death resulted from inactivation of a single site (target) within the cell (Charm 1958; Rahn 1929, 1945; Strumbo 1966). Unfortunately this model cannot describe the non-logarithmic cases, nor sub-lethal bacterial injury effects. However, Moats (1971) described a model in which there are a number of "targets" within the cell and death results from thermal inactivation of a proportion of these sites. This model can be used to describe those cases where there is an initial lag, (A and C in Figure 1.5-1). The curvilinear relationship (D) can be described if the heat resistance of individuals in the population varies. Indeed it is this variation, which is phenotypic and not genetic, that causes the "tails" of reduced killing rate observed in curve C (Moats et al. 1971). The methods of testing thermal resistance and the problems inherent in interpreting the data have been reviewed by Pflug and Schmidt (1971).

WATER

Water Conditions on Milking Machine Surfaces

Milk has a very high water content, so any undried milk soil will have sufficient water for growth. The water conditions on the surfaces of milking machines or any other food equipment have not been investigated. The available water would be expected to be greater in enclosed and poorly drained equipment (e.g. pipelines) than in vessels open to the atmosphere. Where a food soil is present on the

Figure 1.5-1: Four types of relationship between the time of heat application and bacterial survival


Log no. of surviving cells
Time
A
B
C
D

equipment surface, the composition of the soil would greatly affect the water retained in the soil, and also its availability to bacteria on that surface.

Effect of Water on Survival of Bacteria

All organisms require water for life. Micro-organisms can grow only in aqueous solutions, whether the substrate is liquid or solid. The need for a bacterium to survive in environments of varied water content demands a means of preventing the cell contents from equilibrating with the environment. This is achieved by the cell membrane selectively permitting passage of solvents between the cytoplasm and the extracellular environment (osmosis). Because of the higher concentration of solutes inside most micro-organisms compared with that of their environment, there is a spontaneous flow of water from the environment into the organism. The organism competes with the solute molecules for the water it requires for growth. The major role of the strong, non-deformable wall, which encases the majority of bacteria, is to limit the expansion of the protoplast which results from the entry of water into the organism (Rose 1976).

When solutes are dissolved in water there is a loss of entropy and a change in all colligative properties (lowering of the freezing point, raising of the boiling point and a reduction in water vapour pressure). Raoult's Law gives the following relationship for the vapour pressure:

$$\frac{P}{Po} = \frac{n_2}{n_1 + n_2}$$

Where P and Po are the vapour pressures of solution and solvent respectively, and n_1 and n_2 are the numbers of moles of solute and solvent respectively.

Now: $Aw = \frac{P}{PO} = \frac{RH}{100}$

where Aw is water activity of the solution and RH is relative humidity (%).

Also, the relationship between osmotic pressure (OP) and available water is described by:

$$OP = \frac{- RT \log_e Aw}{\bar{V}}$$

where T is absolute temperature and $\bar{V}$ is partial molal volume of water (Scott, 1957).

The minimum Aw values for growth of a range of bacteria are presented in Table 1.5-4. This shows considerable variation between the species of bacteria, although the bacteria in general have higher minimum Aw values than yeast or fungi (Scott 1957). The variation in growth of bacteria in environments of varying Aw is controlled by a number of factors:

1. Each species and strain of organism has an optimum, as well as minimum and maximum water activities for growth (Scott 1957);
2. Oxygen availability - anaerobic conditions raised the minimum Aw for Staphylococcus aureus (Scott 1953) but not facultative anaerobic Salmonella spp. (Christian & Scott 1950);
3. Atmospheric inhibitors - both CO_2 and ozone are more effective inhibitors of growth at low Aw values (Scott 1957);
4. The ability to produce spores - spores are far more resistant to low Aw than vegetative cells (Sykes 1965).

Water Stress on Bacteria in Suspensions

An organism is osmotically stressed when the magnitude of the osmotic pressure difference across the surface layers of the cell is

sufficiently low or high to retard the rate of growth of the organism and affect its ability to reproduce. In hypertonic solutions, water leaves the organism and the plasma membrane separates from the inside of the rigid cell wall (plasmolysis) and collapses around the reduced volume of cellular contents (Rose 1976). This may be followed by a gradual return of the membrane to the inside of the cell wall (deplasmolysis) as low molecular weight compounds pass across the plasma membrane, by a process involving transport proteins (Henneman & Umbreit 1964). Plasmolysis leads to a loss of motility in flagellated organisms (Okrend & Doetsch 1969). This type of osmotic stress can also increase the duration of the lag phase of growth, decrease the growth rate during the logarithmic phase and decrease the mass of cellular material synthesised (Scott 1957).

In hypotonic solutions it is difficult to distinguish between the stress that a bacterium experiences due to high water activity of the suspending solution, and that resulting from being deprived of essential nutrients. Bacteria vary greatly in their ability to survive in hypotonic solutions and their survival can be influenced by temperature, dissolved O_2 tension and the amount of endogenous polymer energy reserves which the cell can use (Rose 1976).

One group of bacteria, the halophiles, has acquired the ability to survive and grow in environments with very high osmotic pressures, by alterations to their physiological processes which cause the magnitude of the osmotic pressure difference across the plasma membrane to be minimised. Halophilic bacteria can grow in almost saturated solutions of NaCl. In fact, they have a minimum requirement for growth of 2.5 - 3.0 M NaCl, the cells containing high NaCl concentrations, conditions to which their enzymes and ribosomes have become

Table 1.5.4: Minimum water activity (Aw) values for growth of a range of bacteria (after Rose 1976)

Organism	Minimum Aw	Reference
Aerobacter aerogenes	0.94	Christian & Scott 1953
Bacillus cereus var *mycoides*	0.99	Burcik 1950
Bacillus subtilis	0.90	Marshall et al. 1971a
Clostridium botulinum Type A	0.93	Marshall et al. 1971a
Lactobacillus viridescens	0.94	Wodzinski & Frazier 1961
Microbacterium sp.	0.94	Brownlie 1966
Micrococcus sp.	0.83	Marshall et al. 1971a
Pseudomonas fluorescens	0.97	Limson & Frazier 1966
Salmonella typhimurium	0.92	Clayson & Blood 1957
Staphylococcus aureus	0.86	Clayson & Blood 1957
Staphylococcus albus	0.88	Marshall et al. 1971a
Vibrio costicolus	0.86	Kushner 1968

uniquely adapted. The halococci have a thick cell wall which enables the bacteria to resist the large osmotic stress of the external suspension. The halobacteria do not have such a thick wall, but have an acidic protein covering the plasma membrane which,in a manner yet unknown,enables them to withstand very high osmotic pressures (Rose 1976).

Water Stress on Bacteria on Surfaces

Very little information is available on the water relations of bacteria on food-contact surfaces. One problem is the difficulty of defining exactly what water conditions exist for an organism. Within the surface of the soil there may be a very high water activity (e.g. Aw 0.99), while a short distance away in the atmosphere the Aw may be low (e.g. 0.30). Thus, at the surface there will be a gradient in Aw which will alter as evaporation continues. It is difficult to identify exactly where the organism is located within this gradient. This is complicated by the changing Aw of both the soil and the air over time (Scott 1957). In milk films on stainless steel surfaces , the time required for a soil-water to equilibrate with the atmospheric humidity increased with both the relative humidity and the depth of soil, ranging from 1 to 10 hours (Barnhart et al. 1970b).

When surface bacteria are exposed to low humidities there is a rapid reduction in cell numbers in the first 2h, an increase in the length of the lag phase of growth and a loss of virulence by pathogenic bacteria (Lowbury & Fox 1953; Maltmann et al. 1960). The ability of bacteria to survive these conditions is dependent upon the vegetative organism involved, its ability to form spores, the initial culture medium, the age of the culture and the sub-culturing conditions (Garvie

1955; Sykes 1965). Death appears to be due to a breakdown of protein structure on desiccation, but it is unlikely that all cellular proteins are equally able to cause death on denaturation, and it is probable that death may result from changes in a few regions of the cell. The lethal changes that occur are associated with the hydrogen bonding which controls the tertiary protein structure (Webb 1960).

On any food-contact surface there is a complex interaction between the types and numbers of organisms, the substrate, the soil, and the atmosphere. In raw milk films on stainless steel surfaces, humidities ranging from 12 - 80% had no differential effect on the bacterial numbers. Under those conditions the bacterial numbers dropped over a 16h incubation period, until the amount of the initial soil was greatly increased (Barnhart et al. 1970b). Wilkinson (1966) studied the survival of bacteria deposited onto stainless steel surfaces from aerosols and found the optimum relative humidity for survival varied greatly with the organism; Serratia marcescens requiring high humidity, Sarcina lutea and Pasteurella pestis low and Pasteurella tularensis a medium level (55%). The survival of Gram negative bacteria on metal surfaces was greater at 11% than 53% which was greater than 85% RH (McDade et al. 1964).

RADIATION

Radiation in Dairy Hygiene

"The amount of microbial destruction by sunlight is enormous and constitutes one of nature's ways of maintaining the balance of life and so of providing a degree of protection from invasion and infection with pathogenic micro-organisms" (Sykes 1965). Despite this there is no deliberate use of the bactericidal effect of sunlight in dairy

hygiene, although the fortuitous contact of this light with some exposed milk-contact surfaces may have some effect. Sunlight contains both visible and ultra-violet (UV) light, and these may both have some effect.

The Effect of Visible Light on the Survival of Bacteria

Visible light up to 580 nm can be slowly lethal by the photodynamic effect (Webb 1961). Visible light can prevent organisms from forming colonies in the presence of oxygen at wavelengths of 365 and 390 - 750, but not at 254 - 313 nm (Webb & Lorenz 1970). The surface colour had no effect on colony formation (Gundermann & Schmehe 1970). The photodynamic action requires the presence of a natural or exogenous photosensitizing pigment, which captures the light energy, which then degrades DNA function (Eisenstark 1971). Many observations of mutagenesis induced by visible light have been reported since that of Kaplan (1950). Non-lethal intensities of visible light increased the mutation rate of E. coli with regard to bacteriophage resistance by 18 times compared to dark conditions (Webb & Malina 1967). Visible light can also induce alterations to membranes, which inhibit the respiration by various bacterial systems. Some of the co-factors in enzymes involved in respiration are sensitive to both near UV and visible light (Krinsky 1976). The permeability of the cell membrane can also be impaired (Mathews & Sistrom 1960). Rountree (1963) reported the lethal effect of sunlight on S. aureus dried on textile surfaces. Dormant spores were more resistant to the lethal effects of light than vegetative cells (Krinsky 1976).

Carotenoid pigments are one of the major protective mechanisms in bacteria for preventing harmful photodynamic effects. The importance

of these pigments has been demonstrated in both photosynthetic and non-photosynthetic bacteria (Konisawa & Stanier 1958; Sistrom et al. 1956). Organisms which normally exist in aerobic environments with the potential for exposure to light would be protected by these pigments (e.g. Micrococcus lutea), whereas organisms without the pigments may be lethally affected (Mathews & Sistrom 1959).

The Effect of Ultra-Violet Light on the Survival of Bacteria

The wavelength of ultra-violet (UV) light ranges from 210 nm to 328 nm, but the most effective bactericidal range is between 240 nm and 280 nm. Most commercial UV lamps utilise a quartz tube which emits its radiation at 254 nm. The lack of penetration of ultra-violet radiation limits the range of applications. In practical circumstances it is not a sterilizing agent - it can reduce but rarely eliminate microbial life. It has a limited range, and long exposure times are necessary (Sykes 1965).

Ultraviolet radiation is absorbed by many cell components, but its mutagenic effects and inhibition of growth result from its interaction with DNA. The chief DNA photo-products formed during UV irradiation are dimers between adjacent pyrimidines and also cytosine hydrate (Bridges 1976). The UV-induced dimers interfere with base-pairing and hence regulation and transcription (Haynes 1964). However, the exact cause of death is often unclear. A number of mechanisms have been reported, including induction of integrated genomes such as prophages, cell wall or cell membrane damage and acute respiratory deficiency (Bridges 1976). The many factors affecting the ability of a bacterium to survive ultra-violet irradiation are presented in Table 1.5-5.

Table 1.5-5: Factors affecting the sensitivity of bacteria to damage by Ultra-Violet light

Factor	Comments	References
The organism	Gram + less sensitive; species and strain variation.	Bridges 1976 Sykes 1965
Growth phase	Most sensitive early lag phase and stationary phase.	Ramage & Nakamura 1962
Protection	Thick cell wall and extracellular materials are protective.	Sykes 1965
Adaption	Sub-lethal exposure temporarily increases resistance.	Luckiesh & Knowles 1948
Chemical protective agents	Glycerol and thiourea scavenge free radicals produced by radiolysis, or react with free radicals on macromolecules. Others bind to DNA, act as energy sinks.	Moroson & Quintiliani 1970; Lion 1976; Bridges 1971
Water content of cell	Drying increases sensitivity	Webb 1965
Temperature	Decreasing temperature reduces sensitivity	Webb et al. 1958
DNA repair mechanisms	1. Photoreactivation - reversal of UV damage by post-irradiation exposure to visible light	Rupert 1960
	2. Excision repair of DNA	Bridges 1976
	3. Rec^{A+} - dependent repair	Bridges 1976
Sporulation	Spores are more resistant than vegetative cells. Sensitivity varies with stage of sporulation, organism and temperature	Russell 1971

VACUUM

In the milking machine, bacteria may be exposed to a vacuum of 50 kPa (380 mm Hg) which exists during the milking, and often during the cleaning phases. Sykes (1965) stated that "vacuum of itself is neither bactericidal or bacteriostatic. Its only contribution to such activity is in depriving aerobic organisms of their necessary oxygen, or in allowing gaseous germicides to penetrate more readily". However, at the level of vacuum in milking machines this effect would be insignificant.

SUBSTRATE AND SOIL

The chemical nature and topography of both the substrate and the soil would be expected to influence the ability of bacteria to survive and grow on them. However, no studies appear to have been reported on this topic.

CLEANING SOLUTION FLOW

Bacteria on the substrate, and on or within a soil, may be removed from the equipment surface by the kinetic energy of the cleaning solution applied as a flowing or impinging fluid. This may be one of the most important contributions to the reduction of bacterial numbers on the surface, but, its role has not been studied at all.

BACTERIAL EFFECT ON SURFACES AND SOIL

Bacterial activity while on equipment surfaces can significantly alter the surface and the soil. The corrosion of metals by bacterial action has been reviewed by McDougall (1966). The magnitude of the problem varies with the type of metal, the availability of oxygen and the type of soil and cleaning solutions, but it does not appear

to be significant where stainless steel is the major metal used. Bacteria can have a far more significant effect on non-metallic surfaces, playing an important role in the degradation of rubber (Berridge 1951; Calderon & Staffeldt 1965; Sphar 1966). The action of bacteria would also be expected to change the chemical and physical nature of the soil, in a similar manner to the way in which dental bacteria influence plaque formation on teeth. However, this has not yet been studied.

CHEMICAL AGENTS

Sanitizing Milking Machines

A sanitizer is "an agent that reduces the bacterial count to safe levels, as may be judged by public health requirements, on food-handling equipment" (Dubois 1949). These materials may be applied alone or in combination with detergents as detergent-sanitizers. The great variety of sanitizing techniques applied to milking machines has been reviewed by Clegg & Cousins (1968). The cleaning systems include; those where separate detergent and sanitizers are used, those where a combined detergent-sanitizer is followed by a sanitizer, and those where the detergent-sanitizer is followed by rinse. The sanitizer has been applied for short contact-times, either after washing, or before the next use. Detergent-sanitizers have been left in contact with the surfaces between milkings to provide a long-contact time soak. On farms, the storage vats and minor utensils are also usually chemically sanitized.

The Nature of Chemical Action on Bacteria

All chemicals have some antibacterial action, are toxic in very high concentrations, and harmless at very low concentrations.

Chemicals classed as "antimicrobials" are those whose relative killing efficiency is demonstrable at low concentrations. All antimicrobial action, even physical methods, owe their effects to chemical changes. All microbial life depends on a complex system of enzymes which may be damaged in many ways. Some of the enzymes are on the exterior of the cell, which exposes them to agents in the medium (Elek 1968).

As the concentration of a sanitizer is reduced, its lethal activity (bactericidal action) becomes attenuated until it is merely inhibitory (bacteriostatic). In most cases the bacteriostatic and bactericidal actions are quite separate phenomena (Roberts & Rahn 1946). Bacteriostasis may be due to reversible inactivation of enzymes, disruption of membranes, or interference with cell synthesis or division (Rahn & van Eseltine 1947). However, in some cases the only difference between bacteriostatic and bactericidal action is a quantitative one (Dubos 1945). These two phenomena cannot be separated into two defined stages - there is transition and overlap which can result in both occurring to cells within a population at one time (Sykes 1965).

All sanitizers are selective in their effect on a mixed bacterial population. The complement of enzymes varies tremendously between organisms and, as the enzymes are a primary site of cellular damage by a sanitizer, variation in bactericidal action results. The lipid content of cells and the composition of cell walls both vary greatly, conferring further variation in the survival of sanitizing. Finally, even in a pure culture of bacterial cells there is a wide range of physiological attributes among the cells, which again affects their ability to survive the lethal action of sanitizing compounds (Elek 1968; Sykes 1965).

The variation in natural resistance of organisms to a sanitizer may have arisen in a variety of ways. Some organisms have acquired resistance incidental to the acquisition of other characteristics valuable to survival, while others result from selection to survive in adverse environments. Organisms carry many enzymes that are usually inactive, but whose actions are "induced" by suitable substrates (Wyss 1968).

Many organisms are able to increase their resistance to a sanitizer when exposed to it for some time. This adaption can result from (Wyss 1968):

1. Cytological changes;
2. Decreased penetration of the sanitzer;
3. Increased destruction of the sanitizer (or conversion to an inactive compound);
4. Increased concentration of a metabolite antagonising the sanitizer;
5. Increased concentration of an enzyme utilizing this metabolite;
6. Decreased quantitative requirement for a product of the metabolite; or
7. Enzyme changes in some quality, such as the relative affinity for the sanitizer.

The most important factor influencing the bactericidal action of a sanitizer is the chemical constitution and configuration of the active ingredients; the activity within many classes of compounds varying greatly with small structural changes (Sykes 1965). The other ingredients in the formulation of the sanitizer will also greatly influence performance, and this will be discussed for each sanitizer below.

The many physical factors that influence the activity of a

sanitizer have been reviewed by Kostenbauder (1968). These include:

1. Solvent effects - solvents may have inherent antibacterial action themselves, or may affect the thermodynamic activity of the sanitizing agent;
2. pH - this may affect the sanitizing action of H^+ or OH^- ions at the pH extremes, or through influencing the species of the sanitizer available at any time;
3. Electrolytes - the ionic environment may affect bacteria through specific ion effects, osmotic effects, or alteration of the thermodynamic activity of the sanitizer;
4. Complex formation and adsorption - complex formation with the sanitizing agent decreases its thermodynamic activity. The following situations have been described: complexes with small molecules, with ions, with macro-molecules, or adsorption to solids;
5. Surfactants - these can assist the sanitizing agent to contact the organisms. The effect can be influenced by micellar solubilisation of non-surfactant sanitizers or self-micellisation of surfactant sanitizers such as QACs(quaternary ammonium compounds);
6. Distribution between immiscible liquid phases;
7. Interfacial phenomena;
8. The capacity of the sanitizing system; and
9. Temperature - change of sanitizing activity is a resultant of changes of rate of growth, rate of thermal death and rate of chemical sanitizing, and can only be predicted over relatively narrow ranges.

The bactericidal activity of most sanitizers is reduced by the presence of organic matter in the medium, and this occurs in most practical situations. The types of interaction which may occur between

the sanitizer and organic matter are listed below (Sykes 1965):

1. The sanitizer may react chemically with the organic matter to give a non-bactericidal complex - reducing the concentration of active sanitizer;
2. The sanitizer may form an insoluble compound with the organic matter;
3. The sanitizer may adsorb to particulate and colloidal organic matter;
4. The sanitizer may be dissolved in fats or phospholipids; or
5. The sanitizer's access to the cell surface may be limited by a layer of organic matter.

The relative importance of these mechanisms will vary with the particular sanitizing situation.

The dynamics of cell destruction have been studied since the 19th century, but a generally accepted description has not yet been developed. Chick (1908) observed that the logarithms of the numbers of surviving organisms were directly related to the times of treatment and stated that "the curves appeared to be very similar in form to that expressing the course of a unimolecular reaction". This could be described by the equation:

$$\frac{1}{t_2 - t_1} \quad \log \frac{n_1}{n_2} = K$$

where n_1 and n_2 are the numbers of bacteria surviving after times t_1 and t_2 respectively (Sykes 1965).

Unfortunately, as with heat sanitizing, chemical sanitizing has produced survival curves which deviate from the logarithmic straight line (Figure 1.5-1). The kinetics of sanitizinghave been reviewed by Prokop and Humphrey (1970). They have reported seven non-logarithmic

models based on the mass action - deterministic approach and five non-logarithmic models based on the probabilistic approach. Almost all of the dynamic work has been done on organisms in suspension. However, Hunt and Pittillo (1967) observed a linear logarithmic rate of kill with bacteria in surface colonies.

As stated above, the effect of temperature on the action of a sanitizer is a resultant of a number of factors. Consequently, there is no simple way of estimating temperature characteristics - they must be determined experimentally over the desired temperature range:

$$\text{The temperature coefficient (per } 10^{\circ}\text{C rise)} = \frac{tx}{tx + 10}$$

where tx is the initial temperature.

The temperature coefficient is an exponential factor, therefore the changes are logarithmic and not directly proportional (Sykes 1965). The temperature coefficient for any sanitizer/bacteria interaction changes over large temperature ranges in a manner described by the Pearl-Verhaulst logarithmic equation (Pearl 1930). The activities of most sanitizers increase with increasing temperature, although there are some exceptions.

The concentration of a sanitizer always influences its activity. Watson (1908) described the exponential relationship:

$$n \log c + \log t = K$$

where t is the time of disinfection, c is concentration of sanitizer and n is the concentration exponent (dilution coefficient).

This relationship is most accurate when the time t is applied to very high kills, (Jordan and Jacobs 1944). The concentration exponent (n) varies with the organism used (Tilley 1939).

The "Ideal Sanitizer"

Pelczar & Reid (1972) summarised the characteristics of the "ideal sanitizer" as:

1. Toxicity to micro-organisms - should have broad spectrum of activity at low concentration;
2. Solubility in water;
3. Stability in storage;
4. Non-toxicity to man and other animals;
5. Homogeneity - uniform composition for equal applications;
6. Capacity to avoid combination with extraneous organic matter;
7. Toxicity to micro-organisms at ambient temperature;
8. Capacity to penetrate materials on surfaces;
9. Non-corroding and non-staining;
10. Deodorising;
11. Detergent ability;
12. Economical.

There is no single "ideal sanitizer" which is the best for all purposes. However, the requirements for sanitizing food equipment have limited the range of chemical sanitizers used. These are discussed below.

Chlorine Compounds

Chlorine-containing sanitizers have been used in the food industry for the longest period. When elemental chlorine or hypochlorites are dissolved in water the chlorine may exist in three forms (Marks et al. 1945).

$$Cl_2 \rightleftharpoons HOCl \rightleftharpoons OCl$$

pH 1 pH 4-5.8 pH 9.3

Of these three forms the undissociated hypochlorous acid is the most

active, being approximately 80 times as active as the OCL^- ion (Fair et al. 1948). The equilibrium between HOCl and OCl^- ensures that the concentration of HOCl is maintained, although HOCl is consumed in killing bacteria (Baker 1959).

The mode of bactericidal action of chlorine on vegetative cells is probably due to the inhibition of enzyme systems through oxidation of the SH groups (Friberg 1956; Green & Stumpf 1946; Knox et al. 1948). Although chloramine compounds are formed by reaction with cytoplasmic proteins, and this can cause breaks in, and chlorination of, DNA (Lu Shih & Lederberg 1976), this does not appear to be the primary cause of death. The primary lethal effect of chlorine on spores appears to be the modification of the outer spore coats, which leads to a disruption of the normal permeability barriers (Kulikovsky et al. 1975). This effect may be enhanced by chlorine stimulating the germination of the spore and then killing the germinated organism (Wyatt and Waites 1973). The influences of the many factors which affect the bactericidal activity of chlorine sanitizers are presented in Table 1.5-6.

Many chlorine compounds, both organic and inorganic, have been developed as sanitizers (Dychdala 1968b). The inorganic group includes sodium, lithium and calcium hypochlorites, chlorinated trisodium phosphate and chlorine dioxide. Of these, the hypochlorites are, by far, the most popular as they are bactericidally very effective, non-toxic to man at the concentrations used industrially, colourless, non-staining, free of residues and economical to use (Lesser 1949). Except for the chloro-phosphates, surfactants cannot be added to them as the bactericidal activity is reduced and corrosion of metals increased (Keogh & Hedrick 1971).

The organic chlorine compounds are the N-chloro derivatives of four groups (Dychdala 1968b):

1. Sulphonamides (Chloramine-T, Dichloramine-T, Chloramine-B and Halazone);
2. Heterocyclic compounds with nitrogen in the ring (Hydantoin, Succinchloramide, Dichloro-and Trichloro-isocyanurates, Trichloro-melamine);
3. Condensed amines from guanidine derivitives (Chloroazodin);
4. Anilides.

These compounds suffer from three major disadvantages: their instability in solution limits them to solid formations, and they are destabilized by surfactants (Davis 1968). They are also more corrosive to stainless steel (Botham 1962).

The uses of chlorine sanitizers in the food industry have been reviewed by Mercer & Somers (1957). In dairy hygiene the early recommendations in the USA were for 50 - 100 mg available Cl (Av. Cl)/l water for at least 10 sec (Prucha 1929). The US Public Health Service later altered this to at least 50 mg Av. Cl/l for a minimum of one minute at 24°C (Dychdala 1968b). In the UK, the recommendation was considerably higher at 250 mg Av. Cl/l at 49°C for 2 min. (Scarlett 1962).

The chlorine sanitizers are corrosive to stainless steel surfaces and promote the deterioration of rubberware (Clegg 1962; Major 1962). They sanitize organisms effectively on hard surfaces, but not porous surfaces such as rubber (Major 1962b). Although hypochlorite solutions lose their bactericidal activity at high pH on organisms in suspension, the activity is undiminished from pH 7 - 11 on organisms on milk-soiled surfaces. This may result from the strong local buffering of the partly-hydrated milk films (Neave and Hoy 1947). The chlorine action is

Table 1.5-6: Factors affecting the bactericidal activity of chlorine sanitizers

Factors	Comments	References
pH	Predominant chlorine species varies with pH - optimal in neutral range.	Fair et al. 1948
Concentration	Fourfold concentration increase reduces killing time on spores by 50%.	Rudolph & Levine 1941
Temperature	10°C rise gives 20-40% increase in bactericidal activity.	Allen 1950
Organic matter	Inactivated by organic matter, but not as much as some other sanitizers. Can degrade some types of organic matter and kill enclosed bacteria.	Johns 1948
Presence of ammonia or amino compounds	Forms chloramines which have slight bactericidal effect.	Cox & Whitehead 1949
The organism	Gram negative less resistant than Staphylococcus spp.,	Johns 1948
	Spore forming organisms 10 - 1000 x as resistant as vegetative organisms.	Tonney et al. 1928

very rapid, no significant persistence of bactericidal effect being observed over a 4h period at the recommended concentrations (Legget 1969).

Bromine Compounds

Bromine compounds have rarely been used as the sole active ingredient in dairy equipment sanitizers, but mainly in mixtures with chlorine compounds. Comparison of the changes in major bromine species in aqueous solutions with pH (presented below) with those of chlorine (presented above), show the hypobromous acid, the bactericidal agent is more available at alkaline pH.

$$Br_2 \rightleftharpoons HOBr \rightleftharpoons BrO^-$$

pH 3 pH 6-8 pH 10.5

Bromine is a more effective bactericide at alkaline pH, its efficiency rising with pH, without any greater corrosion than chlorine to stainless steel (Kristoffersen 1958). However, the cost of bromine compounds has prevented their adoption as the sole active agents in sanitizers.

Iodophors

Iodophors are materials in which iodine is bound to a surface active agent in a concentrated form and released when diluted with water. Their chemistry, bactericidal action and practical applications have been reviewed by Twomey (1968-1969) and Davis (1962). Although many kinds of surfactants may be used, the nonionic surfactants are most popular because they are less affected by pH and water hardness. The iodine is complexed with the surfactant by a hot or cold process. As low pH favours both the bactericidal power and limits the iodide formation, acids, commonly phosphoric, are also added. Dilution of the iodophor in water causes micelle dispersion and dissociation of the

iodine from the surfactant. At the use dilution the concentration of iodine is sufficiently low to dissolve in water. The amount of dissociationis related to the logarithm of the dilution and the surfactants used. The degree of dissociation controls the bactericidal activity.

The active agent in iodophors is elemental iodine,which acts through the halogenation and oxidation of susceptible groups,such as sulphydryls, within cells (Twomey 1968 - 1969). Iodine is a powerful bactericide, being equally effective against Gram positive and Gram negative bacteria (Bartlett & Schmidt 1957; Gershenfeld 1957). It has a bactericidal action only, bacteriostasis not being observed (Truman 1971). Adaptive resistance to iodophors has not been demonstrated (Heather & Vanderzant 1958). On a molar basis the bactericidal effectiveness is approximately 30 times that of chlorine and many more than that of QAC's (Davis 1968).

The bactericidal activity is influenced by a number of factors:

1. pH - the activity increases as pH falls, although the change is far less than that of chlorine;
2. Concentration;
3. Temperature - they have a low temperature coefficient, being one quarter as active at 2 - 5°C as at 20 - 26°C (Chambers et al. 1952);
4. Organic matter - this only has a marginal effect, as long as the pH does not exceed 4 (Davis 1968);
5. Water condition - alkaline, but not hard waters can inactivate the iodophor (Burt & Erwin 1966; Rammell 1966);
6. Spore-forming organisms - iodophors are relatively less effective against spores than are chlorine compounds, based on the concentrations required to kill vegetative cells (Elliker 1954).

There are two types of iodophors; the sanitizer type, which contains just sufficient carrier to hold and stabilise the iodine, and the detergent-sanitizer, which contains additional nonionic surfactant to give the required detergency performance (Davis 1968). The formulation must be prepared so that the iodophor is physically stable on dilution (does not separate into two phases). This can be achieved by careful selection of the combination of surfactants and solubilising agents. Stability of the concentrate during storage is enhanced by incorporation of stabilising agents and storage at low temperatures (Davis 1968). The chemistry of iodophor formulation is discussed by Twomey (1968-1969). The advantages and disadvantages of iodophors are summarised in Table 1.5-7.

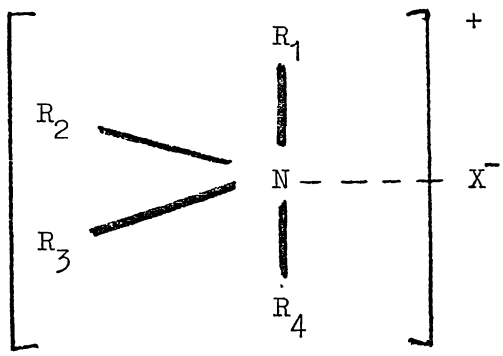
Iodophors have been used as sanitizers on all dairy equipment that can be chemically sanitized. They have also been used as combined detergent-sanitizers for cleaning milking machines (e.g. Hossock 1970). When used for sanitizing, the recommended concentration varies from 12.5 to 100 mg available iodine (Av. I)/l water, but is most commonly 25 mg Av. I/l, while as detergent-sanitizers 50 mg Av. I/l is used (Davis 1968). Iodophors are non-corrosive to stainless steel and, unlike chlorine, do not adversely affect rubberware (Johns 1954, Major 1962b). However, their ability to sanitize older rubberware is not outstanding (Major 1962b).

Quaternary Ammonium Compounds

Quaternary ammonium compounds (QAC's) are substituted ammonium in which the nitrogen atom has a valency of 5. They have the general formula

Table 1.5-7: The advantages and disadvantages of iodophor sanitizers for dairy purposes

Factors		References
Advantages:	Powerful bactericide applied in safe and effective way. Almost odourless. Almost non-irritant to humans and do not cause sensitization. Low toxicity to humans. Combine powerful detergency with effective bactericidal action. Wide spectrum of bactericidal action. Active over a wide pH range. Activity little changed by temperature variation. Less affected by organic matter than other sanitizers. No organisms have developed resistance. Intensity of colour indicates activity. Iodine stains films of organic matter on equipment - indicates areas requiring cleaning. Stability in storage.	Davis 1962
Disadvantages:	Corrosion of metals, staining of skin and expense, if excessive concentrations applied. Lack of persistent antibacterial action on surfaces over extended periods. Possible health problems where excessive residues enter milk.	Leggatt 1969 Dunsmore & Wheeler 1977



Where X is usually a halogen. The bactericidal action is optimal when one of the four organic radicals have a chain length between 8 and 18 carbon atoms. The nigrogen atom may also be part of a ring structure (e.g. pyridine). Many studies show the variation of bactericidal performance with QAC structure (e.g. Cutler et al. 1967; Kondrat'eva et al. 1972; Szego 1966; Verbina et al. 1973).

In reviewing the literature, Hamilton (1971) stated that the unionised base is the active form and that it is the surface activity of the molecule that is important in its bactericidal action. The molecule is "first adsorbed in a biochemically non-specific manner to the cell membrane. Unprotected membranes are sensitive, and non-sensitive organisms are themselves equally sensitive to the adsorption and the action of these antibacterials. Non-sensitivity results from the cell wall, itself either adsorbing or non-adsorbing, acting as a barrier to this adsorption to the membrane. The lesion in membrane function resulting from the adsorption may vary with each antibacterial. With the quaternaries it appears to be a generalised breakdown of the membrane's semi-permeable character". The bactericidal activity of QAC's is greater against Gram positive than Gram negative organisms. This can be explained by the QAC first becoming associated with cell wall protein, and later penetrating to disrupt the cell membrane in the Gram positive organisms; while in the Gram negative organisms the

phospholipids present afford the organism a degree of protection (Blois & Swarbrick 1972).

Many factors influence the bactericidal activity of the QAC's:

1. The organism. As stated above, Gram positive organisms are more susceptible than Gram negative, but spores are not susceptible at all (Hamilton 1971);
2. Organic matter. The active adsorption of the surface-active QAC to organic matter considerably decreases the available concentration and therefore the bactericidal effect;
3. Surfaces. QAC's also adsorb to hard surfaces, which again reduces their bactericidal effect. However, they do provide a persistant antibacterial activity on the surface (Hamilton 1971) and sometimes some residue problems when contaminating food (Feagan & Murnane 1965);
4. pH. Activity is optimal in alkaline pH;
5. Temperature. Activity is increased at higher temperatures (Hamilton 1971;
6. Incompatible materials. The bactericidal performance of QAC's is reduced when they are mixed with any of many incompatible materials. These include anionic surfactants, hardness salts in water, and many other compounds listed by Lawrence (1968b).

QAC's have the advantages of combining reasonable bactericidal activity in an odourless, colourless, stable liquid which does not corrode most metals, is non-toxic to humans and can also act as a detergent. The main disadvantages of QAC's are the limitations in antibacterial spectrum, slower bactericidal action,possible residues and expense (Prince et al. 1961). One further disadvantage is the ability of many organisms to develop a tolerance to concentrations of

QAC in the medium (up to 100,000 mg/l with one organism - Crocker 1951). Soprey & Maxcy (1968) and Maxcy et al. (1971) found E. coli could adapt to 28 mg QAC/l by altering the adsorption of QAC to the cell. They considered the tolerance insignificant in practice, as development of tolerance required: an increasing QAC gradient, growth conditions to permit progeny production, and continuing challenge. Also, the tolerated concentration was well below the concentration of sanitizers normally applied, and the organism grew less vigorously and died off more rapidly. However, Washam et al. (1976a, b, c) studied a strain of Pseudomonas aeruginosa which tolerated 770 mg QAC/l in the medium. This organism did not lose its tolerance when not exposed to QAC, was more resistant to osmotic disruption, but had a reduced metabolism and growth rate. The organism had permanently lost its flagellum and stored materials intracellularly. The QAC affected the cells both extracellularly and intracellularly in genetic alterations.

When QAC's are formulated into detergent-sanitizers the other detergent components can influence the bactericidal activity, detergency and ability to act in hard waters (Cousins & Clegg 1956; Thomas 1961). The conditions of use recommended for sanitizing are usually 200 mg QAC/l at ambient temperature for 15 - 30 min. (Thomas 1961).

When used for milking machine cleaning alkaline QAC detergent-sanitizers initially gave poor detergency and permitted development of Gram negative organisms on machine surfaces (Dahlbert et al. 1950). A different formulation applied at 55°C gave satisfactory detergency, but produced a coliform problem in the milk - which could be overcome by increasing the temperature to 88°C (Twomey, 1966). An acidic QAC

has also given satisfactory performance for milking machine cleaning at 80°C (Heyes and Dunsmore 1978). Rubberware should be replaced regularly as QAC does not efficiently sanitize old rubber (Major 1962b).

Chlorhexidine

Chlorhexidine is the member of the N^1N^5-substituted biguanide series which has the greatest antibacterial activity. Its formula is 1, 6 - di - (4 - chlorophenyldiguanadinohexane), the digluconate salt being freely soluble in water. Chlorhexidine is not surface-active, and where surface activity is required cationic or nonionic surfactants are added; it is incompatible with anionic surfactants or other anionic compounds. The chemistry and bactericidal properties of chlorhexidine have been reviewed by Longworth (1971).

When added to a bacterial medium chlorhexidine immediately adsorbs to the bacterial surface. The degree of adsorption is controlled by the chlorhexidine concentration, cell density and species of organism, and composition and pH of the suspending medium. After adsorption, the cytoplasmic membrane is disrupted. At low concentrations of chlorhexidine this results in leakage of cytoplasmic constituents and, at higher concentrations, in coagulation of the cytoplasmic constituents. Thus, at all concentrations death is caused by leakage resulting from membrane damage (Longworth 1971). The activity of membrane ATPase and the active transport of amino acids are both inhibited (Daltrey & Hugo 1974). Bacteriostasis can be induced by slight membrane damage and enzyme inhibition.

The bactericidal activity is affected by concentration and the ability of the organism to develop tolerance to the sanitizer (Stickler 1974). Chlorhexidine is active against both Gram positive and Gram

negative vegetative organisms, except for acid-fast bacilli; the different susceptibilities being due to varying capacities to adsorb chlorhexidine, or differing resistance to lysis and coagulation. Chlorhexidine is not sporicidal. Chlorhexidine sanitizers are used for post-milking sanitizing of the teats of cows as a mastitis-control procedure, but not often in cleaning systems for milking machines.

Amphoteric Compounds

The amphoteric compounds are high molecular-weight amino acids with the general formula R $NHCH_2$.COOH, where R is usually an alkyl radical, but can be an acyl one. These amphoteric compounds combine detergent properties in the anionic group with bactericidal properties in the cationic group. The two compounds most used in the food industry are "Tego 51" and "51B". Tego 51 contains dodecyldi-(amino ethyl)-glycine hydrochloride and has an optimal pH of 9.1 - 9.4. Tego 51B is based on mixtures of compounds with alkyl radicals of slightly different chain lengths and has an optimal pH in the range 7.5 - 7.7 (Davis 1968; Sykes 1965).

These compounds cause small perforations which develop as tubules into the outer lamellae of the three-layered wall of the bacterial cell. The cell wall then forms extruded vesicles which burst. In older cells the cytoplasmic membrane, and, perhaps the cytoplasm, produce myelin-type structures which fill the cell (Lickfeld 1966).

The amphoterics are claimed to be actively lethal against all bacteria, but their action against the resistant Gram negative organisms is in some doubt (Davis 1968). In terms of bactericidal activity they have the advantages of low toxicity, combined detergent-sanitizer action, and lack of residue problems. However, slow action, and their

expense has precluded their wide adoption (Davis 1968; Lindgren 1953). Tego 51 has been found satisfactory for dairy purposes at 0.2 to 1.0% with a contact time of 5 - 10 minutes in Germany (Roland 1955), Norway (Lindahl 1966), France (Sainclivier & Kerherve 1972), but not Hungary (Wagner et al. 1971). The mixture Tego 51/15 DL was also found to perform satisfactorily (Sipka 1972).

Acids

Both mineral and organic acids can be used as sanitizers, the major ones being nitric, phosphoric, acetic and sulphamic. The mineral acids are active primarily through the concentration of the hydrogen ions (H^+), although the characteristic ions also contribute. The organic acids owe their bactericidal activity primarily to the undissociated molecules (Sykes 1965).

Acids have the advantages of rapid action and detergency of mineral-type films (Davis 1968). Nitric acid is successfully used as a detergent-sanitizer in boiling water for milking machine cleaning (Clough et al. 1965). It has also been used as a cold sanitizer at 0.06N satisfactorily in Denmark, and Eire, but not the UK (Cousins et al. 1966; Palmer 1974; ;Pedersen & Moller-Madsen 1959). Acetic acid was found to be effective and non-selective in the meat processing industry (Anderson et al. 1977). Phosphoric and sulphamic acids are primarily used as detergents, but also have active bactericidal action.

Alkalis

The only alkalis which have any significant bactericidal activity are those in which a 1% solution has a pH greater than 12. Although this includes sodium hydroxide (NaOH), protassium hydroxide (KOH), sodium metasilicate ($Na_2SiO3.5H_2O$), sodium orthosilicate

($2NaO.SiO_2.(5.5)H_2O$) and sodium sesquisilicate ($1.5\text{-}1.6Na_2O.(1)SiO_2.(5.5)H_2O$), in practice the major alkali used is NaOH (Clegg 1970). As the bactericidal activity of these materials is primarily attributable to the hydroxyl ion (OH^-), the bactericidal action is governed by the degree of dissociation (Sykes 1965).

The alkalis combine powerful detergency, through protein hydrolysis and saponification of fats, with effective sanitizing against all bacteria except the acid-fast types (Foster et al. 1958). The combinations of concentrations, temperatures and time required to kill spores has been examined (Franklin & Clegg 1956; Gehuchte 1963; Keogh & Hedrick 1971). Sodium hydroxide causes the superficial layers of the spore to swell and the sporoplasm to leave the cell, with the primary lethal damage being destruction of the cell's permeability barrier (Kulikovsky et al. 1975).

Alkalis have been used on dairy farms for soak cleaning techniques where the contact-time between sanitizer and surface is lengthy. It is applied at 0.5% in the lye-storage method of cleaning pipeline milking machines (O'Callaghan et al. 1965) and at 3% with EDTA for immersion cleaning of bucket milking machines (Thiel et al. 1956). This latter concentration may be raised to 5% to ensure satisfactory control of spores in low ambient temperature conditions (Clegg 1970). The major disadvantages of NaOH are that it is dangerous to users, corrosive to many metals, and ineffective as a detergent against mineral films (Davis 1968).

Acidified Wetting Agents

Acidified wetting agents are mixtures of bactericidal anionic surfactants, nonionic surfactant and phosphoric acid, which have an

operating pH of 2 - 3. Their mode of bactericidal action is uncertain, but there are three possibilities: a general denaturation of cell proteins, inactivation of essential enzymes, and disruption of cell membranes and their permeability barriers (Dychdala 1968a). The factors affecting their bactericidal effectiveness include:

1. The organism - both Gram positive and Gram negative vegetative bacteria are rapidly killed, but spores are resistant (Lewandowski 1952);
2. pH - the optimum range is 1.9 - 2.2 with activity reducing markedly above 3 until minimal activity occurs at pH 7 (Dychdala 1968a);
3. Temperature - increasing temperature improves activity;
4. Anionic surfactant - alkyl aryl sulphonates give maximum activity.

The activity does not appear to be affected by water hardness, organic matter, or inclusion of 1% nonionic surfactant in the formulation (Lewandowski 1952).

Dychdala (1968a) has listed the advantages of the acidified wetting agents as: non-staining, lack of objectionable odour, milkstone and waterstone removal, wide spectrum of bactericidal action, stability in concentrated or diluted solutions, lack of corrosion of stainless steel, and short-duration residual bactericidal activity. Its disadvantages include: bactericidal activity only at acid pH, generation of foam, slow activity against spores, the need for rinsing before the milk contacts sanitized surfaces, and their expense, which has prevented their widespread adoption (Clegg 1970; Dychdala 1968b).

Detergents

The major bacterial-control activity by detergents is physical removal of the organisms. However, many major components also have

significant bactericidal activity. The activity of the acids, and alkalis and cationic surfactants which are incorporated into many detergents have already been discussed above.

The bactericidal activity of surfactants is related to their ability to lower the surface tension of the medium (Maxcy 1964b). The cell is killed by irreversible alteration of the permeability of the osmotic barrier (Obayashi 1965; Wahn & Zapf 1972; Watanabe & Osawa 1966). The antibacterial action of anionic surfactants varies from slight for most compounds,to very active for those used in the acidified-wetting-agent sanitizers (Davis 1978; Kotani et al. 1966). Most anionic surfactants inactivate QAC's and destabilise chlorine-releasing compounds, which prevents their use in these detergent-sanitizers. Nonionic surfactants have a very mild antibacterial action, the activity of which is related to their adsorption to the cell (Kotani et al. 1966; Laminkanra & Allwood 1976). They are widely used in detergents and sanitizers because they are effective detergents and are compatible with most ingredients (Davis 1968).

Ethylenediaminetetra - acetic acid (EDTA) is a common ingredient in detergents because of its chelating action. The sodium salt of EDTA is actively bactericidal (Brown & Richards 1965), by effecting a non-specific increase in cellular permeability (Leive 1965). It is active against both Gram positive and Gram negative bacteria (Russel 1971) and it improves the antibacterial action of QAC's and chlorhexidine (Brown & Richards 1965). Phosphates are also important detergent ingredients. Many of these, particularly the polyphosphates, have significant antibacterial activity. The bactericidal action of the phsophates have been reviewed by Hargreaves et al. (1972).

The Interaction Between Detergency and Sanitizing

Although the toxins from some dead bacteria can be a health problem (e.g. staphylococci and clostridia), in practice the most significant hygiene problems occur when food is contaminated by live bacteria. Consequently, the removal of a live bacterium from a surface is at least as effective a method of bacterial control as the killing of that organism. Thus, detergency must be considered as significant a bacterial control technique as sanitizing.

After a milk soil which contains bacteria has been deposited on equipment surfaces, the organisms may be controlled by removal or kill. If a soil residue remains after cleaning and sanitizing it may support bacterial growth and those organisms may contaminate the milk at the next milking (Clegg 1962 ; Davis 1956a). Davis (1956a) defined a sanitized surface as one which meets the following four criteria:

1. Absence of all soiling matter;
2. Absence of all pathogens;
3. Absence of all fault-producing organisms;
4. A total bacterial count not exceeding one colony per cm^2 or ml capacity.

Inherent in this definition is both the physical removal of the soil and control of the microbial population.

"Bacteria are dirt. In theory it should be possible to 'sanitize' a surface using non-sanitizing detergents" (White 1962). Indeed, as up to 99.8% of soil and bacteria can be removed from a surface by simple cleaning, the detergency of bacteria (bacterial removal) is quantitatively more important than the sanitizing of bacteria (bacterial killing -- Davis 1956a; Hays et al. 1960; Malmann et al. 1953; Mitchell & Clifford 1964). Unfortunately, by using a detergent only, the com-

plete removal of soil and bacteria cannot be achieved (Masurovsky & Jordan 1958), so a sanitizer must also be applied to kill the surviving bacteria. In practice it is often difficult to distinguish between the detergency and the sanitizing performance of a particular solution. All detergents are bactericidal to a degree and all sanitizers will exert some detergent action.

Detergent-sanitizers and Sanitizers

Detergent-sanitizers are mixtures of detergents (surfactants, acids, alkalis, phosphates etc) with sanitizers (chlorine, iodine etc). Detergent-sanitizers both clean and sanitize. The most common detergent-sanitizers are the chlorinated-alkalis, acidic and alkaline QAC's, iodophors, amphoterics and nitric acid (Davis 1968; Heyes & Dunsmore 1978).

The advantages of detergent-sanitizers include:

1. They give an extra margin of safety - bacteria removed from a surface into a detergent solution may remain viable and be redeposited at other sites on the equipment. However, bacteria removed by a detergent-sanitizer would be killed in the solution, and contamination could not take place, even if they were redeposited (Davis 1968);
2. The sanitizer often increases the detergent activity - e.g. chlorine degrades protein (Davis 1968);
3. The combination of detergency and sanitizing in one solution reduces the number of operations required, saving time and money (Davis 1968);
4. The sanitizing performance of the system may be improved - the use of a chlorinated-alkaline detergent for cleaning bucket milking machines reduced the counts over a separate detergent and sanitizer

system. This was attributed to the longer contact time of the chlorine in the detergent, as opposed to the final rinse (Clegg 1967). However, in laboratory tests, even though the contact time was longer for the detergent-sanitizer, the separate detergent and sanitizer treatment was superior (Mitchell & Clifford 1964).

Some of the disadvantages of the use of detergent-sanitizers are:

1. The absence of an effective washing before the sanitizing means that, with the combined detergent sanitizer- the sanitizer must act in a medium which contains a relatively high concentration of organic matter, which may impair its performance;
2. The conditions required for good detergency may result in the sanitizer being used in sub-optimal conditions. Similarly, the sanitizer requirements limit the range of detergent materials that can be used; and
3. The operator may not understand that the product must be applied correctly in terms of the pre-rinsing, post-rinsing temperature concentration, turbulence and contact time necessary to achieve a satisfactory result (Davis 1968).

Factors Affecting Sanitizer Performance in Practice

The performance of a sanitizer in a practical situation are governed by many factors, including:

1. The sanitizer used. The speed, specificity, penetration of soil, wetting of bacteria and soil, and persistence on surfaces after application are all influenced by the type of sanitizer (Clegg 1962; Leggat 1969);
2. The temperature, concentration and contact-time of sanitizer application (Clegg 1962);

3. The surface being sanitized. The material and design, the chemical nature, topography and porosity of the surface, can have a major effect on sanitizing performance (e.g. most chemical sanitizers perform poorly on old rubber surfaces - Major 1962b). Also, corroded and cracked metals cannot be satisfactorily sanitized by chemicals (Clegg 1962; Daufin 1967; Maxcy et al. 1971);
4. The soil on the surface. If the detergent is inefficient and leaves an excessive soil residue, the sanitizer which follows will be partly inactivated by the residual organic matter;
5. Time of sanitizing within the cleaning system. Davis (1968) described the various methods;
   5.1 cleaning by detergent and then sanitizing,
   5.2 cleaning by strong concentration of detergent-sanitizer followed by a weak sanitizer,
   5.3 cleaning and sanitizing by a strong concentration of detergent-sanitizer followed by a "sterile rinse",

   The sanitizer in 5.1 and 5.2 may be applied immediately after the washing, or left until immediately before the next use of the equipment. The use of the pre-product rinse was found to be more efficient in milking machine cleaning (Middleton et al. 1965), and is recommended by many workers (Cleg 1962; Davis 1968; Johns 1962b). In this position in the cleaning system it will sanitize the surface immediately before the passage of the product - just when it is most required. Unfortunately, one result of this is that residues of the sanitizer solution will remain on the equipment surfaces and may contaminate the product. This problem can be overcome by a rinse with water of good quality. However, if the bacteriological quality of the water is poor it may be improved

by the addition of a low concentration of a sanitizer like chlorine or iodine (Hays et al. 1967).

6. Rest period treatment. The treatment of the equipment between the completion of washing and the next use (intercycle period) determines the opportunity any surviving bacteria have of proliferating to a level where they might contaminate the product in significant numbers at the next use. The potential for contamination is influenced by both the length of time this period lasts and the ambient temperature. Davis (1956a) recommended that equipment should be stored in this period, either dry, or in contact with a sanitizer solution of low concentration. Maxcy & Shahani (1960) achieved very satisfactory results with a low chlorine concentration in a pipeline system.
7. Application method. The major methods of applying the sanitizing solution to milking equipment are: circulation through pipelines and small volume equipment, immersion of small items and brushing of open equipment (Rice 1962).
8. Periodic treatment. Whenever a cleaning and sanitizing system is applied repetitively to equipment, a soil which is resistant to that system may accumulate. The inability of chemical sanitizers to penetrate this film has led to recommendations for a periodic treatment. The use of an alternative detergent treatment which will remove the scale (e.g. acidic detergent to remove mineral soil left by routine alkaline detergents) is the best technique, as the harbourage for the organisms is removed (Clegg 1962; Davis 1968; Whittlestone & Phillips 1959). However, some suggest that a periodic heat treatment by steam or boiling water, which will kill the bacteria within the soil without removing the soil itself, will be sufficient

(Clegg 1962; Cuthbert 1961; Davis 1968). This can only be effective over a short period as the soil remains and will again permit bacterial colonisation.

9. The operator applying the sanitizer. The success of sanitizing is dependent on the operator understanding the task and being sufficiently motivated to routinely apply a simple technique in an effective manner. The best technology is useless if it is incorrectly applied (Clegg 1962).

INJURY OF BACTERIA

Bacteria are injured when, after surviving a particular stress, they lose some of their growth characteristics. The injured cells are damaged but have the capability to function in an unrestricted environment and restore a normal physiological state concomitant with the initiation of growth and cell division (Busta 1976).

The many treatments which are applied in dairy hygiene to control the growth and survival of bacteria have been described above. These treatments include heat, cold, freezing/thawing, desiccation, nutritional stress, irradiation and detergents and sanitizers. Most of the treatments which have the ability to destroy an organism also have the potential to damage the cell when applied at sub-lethal levels (Busta 1976).

It is not yet known whether different stresses cause injuries at different primary sites, or whether there is a common site, although the latter seems unlikely. All forms of injury involve membrane damage with loss of internal solutes and increased sensitivity to unfavourable chemicals. Changes in cell wall, cell membrane, ribosomes, chromosomes and metabolism have all been observed. Some of these

changes may be the primary cause of injury and the others secondary (Hurst 1977). Spore injury can result from damage to the germination system, damage during the formation of the delicate first vegetative cell, or during outgrowth (Hurst 1977).

Many factors have been shown to influence the degree of injury, including:

1. The growth conditions which affect the physiological state and composition of the cell.
2. The factors which affect cell quality - not only do these affect the survival of the stress, but the organisms' ability to repair the damage.
3. Antagonistic agents have potentially greater activity against damaged cells, (Busta 1976).

Injured bacteria generally display an inability to proliferate under specific conditions that were previously productive. They may lose their ability to form colonies or turbid suspensions, lose their resistance to selective agents,and have a prolonged lag phase of growth (Busta 1976; Hurst 1977). Their nutritional requirements are often more exacting, but that is not always the case (Hurst 1977).

The existence of injured cells can be very significant in dairy hygiene. Injured cells in the milk pose a hazard as they may be capable of repair and toxin production (Collins-Thompson et al. 1973). Also, the inability of conventional detection and counting methods to include injured cells means that the effectiveness of any control treatment may be over-estimated when measured using the conventional techniques employing indicator organisms (Speck 1970). Sublethal injury may also act as a selection process which would eliminate sensitive forms and promote the domination by forms resistant to the stress (Busta 1976).

1.5.4 BACTERIAL GROWTH ON SURFACES

For an organism to survive and grow on the surfaces of food equipment it must have the appropriate physical, chemical and biological micro-environment. Just what constitutes these "appropriate" conditions are discussed in this section.

PHYSICAL REQUIREMENTS FOR GROWTH

Temperature Requirements for Growth

Temperature is one of the most important factors influencing the growth and survival of bacteria. As temperature rises the rates of a cell's chemical and enzymatic reactions increase. However, a limiting temperature is reached where inactivation occurs, giving each organism a minimum, optimum and maximum temperature for growth, or any particular cellular activity. Bacteria vary widely in their temperature characteristics and have arbitrarily been divided into:

1. Psychrotrophs - those able to grow at 0°C (although they may have optima at 20 to 25°C);
2. Mesophiles - those with temperature optima in the range 25 to 40°C;
3. Thermophiles - those growing at temperatures greater than 45°C (Brock 1970).

The distribution of organisms within those classes is not readily altered by selection (Farrell & Rose 1967a).

These classes of organisms vary greatly in their ability to grow at low temperatures; as the temperature is reduced psychrotrophs will grow until the medium freezes, while mesophiles will not (Hagen 1971). This ability has been associated with a number of biochemical features of these organisms:

1. The greater activity of enzymes from psychrotrophic bacteria at

lower temperatures (Ingraham 1958; Saltzer 1961), resulting from lower activation energies (Burton & Morita 1963).

2. Avoiding metabolic imbalances resulting from distortion of the complex balance between induction and regression of the synthesis of enzymes,and the end-product inhibition of the activities of synthesised enzymes (Hagen 1971).
3. Superior cellular permeability at low temperatures by having different permeases or differing membrane structure (Bishop & Still 1963; Farrell & Rose 1967a, b; Hagen 1971).
4. Prevention of the uncoupling of energy production from energy utilisation (Ng 1969).

There are also cellular biochemical characteristics which permit some organisms to function at elevated temperatures. The fact that the macro-molecules of thermophilic bacteria are relatively heat stable is apparently a key to their heat stability (Campbell & Pace 1968). This stability may be an intrinsic property of these macromolecules or due to the presence of protective substances within the cells. It has also been suggested that the variations in the temperature at which membrane lipids solidify, and also the rapid resynthesis of denatured proteins play a role, but their importance appears to be minor (Gaughran 1947 a, b; Allen 1950).

A "limiting factor" is any variable in a micro-environment which controls the distribution or density of a population (Alexander 1971). Temperature is an important limiting factor on any dairy equipment surface. The feature of temperature which are important are the duration and range of the extremes and any regular or irregular variation which occurs. The organism which can survive and grow quickly under the particular conditions of a site will be at a competitive advantage.

Water Requirements for Growth

Water is essential for life. The influence of the water available in the micro-environment on the survival of the organisms in that environment have already been discussed in Section 1.5.3. This demonstrated that, although bacteria can survive for some time within low moisture conditions, they require high moisture conditions for growth.

In the milking machine there are two types of surface situation:

1. The thin film. Here the equipment surface is smooth and unmarked and is exposed to the turbulent cleaning solution. There is an even film of thin soil.
2. The "harbourage" (Maxcy 1973). Here the equipment surface is cracked or corroded, or assembled so that there are crevices between components. There is an accumulation of a mass of soil within the surface imperfections.

Where there is a thin film the bacteria are well exposed to the detergents and sanitizers of the cleaning system. Also, the water content of the film would drop quickly after cleaning to equilibrate with the humidity of the atmosphere over the soil (Barnhart et al. 1970b). If the atmospheric humidity is below 80% RH there would be insufficient water available to the bacteria in the film to grow.

Bacteria within a "harbourage" are embedded in a nutritive medium. They are protected from the chemical action of the detergents and sanitizers. Also the surface area : volume ratio of the soil is low, compared to that of the thin film. This limits the rate of water loss from the soil, possibly to the point where the time for which there is sufficient water for growth exceeds the lag time of the organisms, and growth occurs.

Maxcy (1973) showed that growth in a harbourage maintained at 80% RH

was equivalent to a thin film situation maintained at 100% RH. The relative humidity also exerted a selective effect on the milk-derived organisms growing on the surfaces. At 80% RH Gram positive organisms dominated the microflora (streptococci, micrococci and Gram positive rods), while at 100% RH the microflora was dominated by Gram negatives (coliforms, other Gram negative rods). Humidities between 86 and 93% RH did not exert a selective effect on the microflora.

pH Requirements for Growth

All bacteria have their characteristic minimum, optimum and maximum pH values for growth, and most, but not all, grow in neutral or mildly alkaline conditions. Data for a representative range of organisms are presented in Table 1.5-8. However, despite this variation in the pH of the external medium the pH within the cell is maintained near neutrality, by either preventing H^+ ions from entering,or actively excreting H^+ ions as rapidly as they enter. The pH also effects the ionisation of both the cells and solutes in the medium. The permeability of most solutes is optimal in the non-ionised form - which is pH-controlled (Brock 1970). The pH of milk films on surfaces has not been examined.

Eh Requirements for Growth

The oxidation-reduction or redox potential (Eh) of a micro-environment is one measure of its chemical nature - its ability to donate or accept electrons. In biological systems the redox potential is controlled by the concentration of oxygen in the following way:

$$Eh = 1.234 - 0.058\ pH + 0.0145 \log pO$$

where pO = partial pressure of oxygen (Brock 1966).

Oxygen has profound effect upon the oxidation-reduction reactions

within bacteria. A "typical" anaerobe might grow in the range -150 down to -420 mV, while aerobic cultures may range from +300 mV down to -200 mV, or -420 mV if they produce hydrogen (as many facultative anaerobes do, Hewitt 1950). It seems likely that the extracellular components of a culture are in equilibrium with the metabolic pool inside the cell, and that changes in external Eh are reflections of, or are reflected by, the redox state of this pool" (Wimpenny 1969). The changes in Eh of films on hard surfaces have been determined for some microenvironments (e.g. teeth - Russell & Coulter 1977), but not for those in milking machines.

CHEMICAL REQUIREMENTS FOR GROWTH

Nutrient Requirements for Growth

The nutrient requirements of bacteria range from the very simple to extremely complex. The necessary nutrients must be available at a concentration within the correct range for growth to occur: too low and growth will not occur; too high and toxicity can cause death. Where an organism is supplied with two functionally equivalent nutrients the material it utilizes first is that on which it has to do the least amount of work for macromolecular synthesis or metabolic function (Brock 1966). Discussions of the nutrient requirements of bacteria are available in any standard microbiological texts (e.g. Brock 1970).

All of the bacteria colonising food equipment surfaces are chemo-organotrophs (chemotrophic and heterotrophic) obtaining all of their energy from organic compounds. The majority will be aerobic, or facultative anaerobes, although a few strict anaerobes may be buried under the soil in heavily-soiled sites. The amount of protoplasm an organism can produce is proportional to the energy available to it

Table 1.5-8: Data on minimum, optimum and maximum pH for growth of a selection of bacteria (from Kushner (1971)

Organism	pH		
	Minimum	Optimum	Maximum
Diplococcus pneumoniae	7.0	7.8	8.3
Strep. pyogenes	4.5	7.8	9.2
E. coli	4.3	6.0 - 7.0	9.5
Sal. typhosa	4.0	6.8 - 7.2	9.6
Pr. vulgaris	4.4	6.5	9.2
P. aeruginosa	4.4	6.6 - 7.0	8.8
B. subtilis	4.5	6.0 - 7.5	8.5
Cl. sporogenes	5.0	6.5 - 7.5	9.0
Th. thiooxidans	1.0	2.0 - 5.0	9.8
Mycobacterium tuberculosis	5.0	6.8 - 7.7	8.4
Ser. marcescens	4.6	6.0 - 7.0	8.0
	4.0		9.0
Acetobacter acidophilum	2.8	3.0	4.3

from a substrate. However, the available energy does not influence the growth rate significantly, this being more controlled by intrinsic factors of the organism and physical and chemical factors in the environment. The variation in energy-conversion efficiency between organisms is determined genotypically (Gunsalus & Shuster 1961).

Dairy Soils as Growth Media

Considering the importance of bacterial contamination of foods to the food industry, remarkably little work has been performed on the effect of chemical composition of foods and food residues on bacterial growth. Many foods are very good bacterial growth media. Milk contains a good complement of carbohydrate (lactose), proteins (casein, whey proteins), small peptides and free amino acids, non-protein nitrogen and triglycerides, free fatty acids, phospholipids and sterols, the water-soluble B vitamins and an adequate complement of inorganic ions. It may also contain some natural materials which may inhibit bacterial growth (e.g. lactenins and cationic proteins - MacMillen & Hibbit 1972; Reiter & Bramley 1975) or antibiotics and sanitizers resulting from contamination, which have similar effects (Foster et al. 1958). Milk and milk products contain the nutrients required by many organisms.

Of the organisms studied by Gibson & Abd-el-Malek (1957), those which grew in milk included: Streptococcus lactis, Leuconostoc spp., staphylococci and Gram negative rods. Modifications to milk can greatly widen the range of the organisms it will support (Foster et al. 1958).

When milk is placed on a surface, dried to a film, and then washed, the composition differs greatly from that of the original milk, and so

do the organisms that the film will support. The major limitation is the water available, and this has been discussed above. The observation that a minimum amount of soil must be available on the surface before growth occurs (Bacic & Clegg 1967; Barnhart et al. 1970a), is probably a reflection of the water requirement, although it could also be a nutrient effect. Modification of the composition of the milk film (e.g. addition of milk solids or phospholipids) can markedly alter the ability of organisms to grow (Chaturvedi & Maxcy 1969; Maxcy 1964a, 1973).

The studies of (Gill 1976) on the colonisation of meat surfaces showed that in an aerobic environment a Pseudomonas sp initially used the available glucose, but when this was exhausted, or available at a rate too slow to maintain the growth rate, the organism used amino acids and lactate. Growth was limited by either lack of O_2 or accumulation of toxic materials. This type of study should be performed on a wide range of food soils, particularly milk films on equipment surfaces.

Starvation

The majority of organisms deposited onto the surfaces of food equipment probably occupy sites where there are few if any nutrients. They may suffer starvation, but this has not been studied under these conditions. The majority of bacteria can survive, sometimes for considerable periods, in the absence of nutrients. The ability of a cell to survive starvation is dependent upon the growth phase and growth rate when starved, nutritional status, population density, biological history and the nature of the starvation environment (Dawes 1976).

Cellular processes, including osmotic regulation, maintenance of

intracellular pH, turnover of macromolecules and motility, require energy. When a cell is starved this energy must be supplied from endogenous sources. The "energy of maintenance" is that consumed for purposes other than production of new cell material (Dawes 1976). Approximately half the maintenance energy is required for resynthesis of protein and RNA during turnover (Marr et al. 1963), and much of the remainder is used for stabilisation of ionic composition and intracellular pH (Stouthamer & Bettenhausen 1973). The ability of a cell to survive starvation will, to a large extent, reflect its maintenance of a low rate of endogenous metabolism, matched to the maintenance energy requirements (Thomas & Batt 1969).

There are four major possible sources of substrates for endogenous metabolism. Up to one half of the free amino acid pool of Sarcina lutea was oxidised during 5h starvation (Dawes & Holms 1958). The intracellular proteins are the second source, proteolysis being more generalised and uniform in starved cells (Pine 1972). RNA may be degraded to ribose, purines and pyrimidines which, depending on the organism, may or may not be used as endogenous substrates. However, in starving bacteria there may be many times the necessary amount of RNA without this being utilised (Dawes 1976). The magnesium content of the cell greatly influences survival following starvation. Intracellular reserves of glycogen, lipid or polyphosphate may be used as endogenous substrates in some cells, but this is not always so (Dawes 1976).

BIOLOGICAL FEATURES OF GROWTH

The Nature of Bacterial Growth on Surfaces

On solid surfaces, where motility and Brownian motion are impaired,

the growth and division of cells produces a very high concentration of cells - a colony. "A colony is more than a mere collection of cells all in one place. A colony has shape, form, texture, size, structure and colour. It is the collective expression of the wonderfully complex processes of cell growth, division and interaction" (Brock 1966).

Not all cells in a population are identical. Legroux and Magrou (1920) postulated that a colony is a biological entity which undergoes differentiation in a manner analagous to higher organisms, and this view persists today (e.g. Kauffmann & Letelier 1971). This type of differentiation is displayed most obviously in those colonies containing macroformations such as fruiting bodies, swarms, aggregates and endospores. This heterogeneity of individuals within a population is also demonstrated in the performance of these organisms under a wide variety of conditions, such as disinfection treatment or cold shock (Brock 1966).

The macroscopic morphology is extremely diverse with regard to size, structures, periphery, colour and texture. These macroscopic features reflect the available nutrients, the genetic complement of the cells (e.g. rough vs smooth colonies from unencapsulated and capsulated cells - Thimann 1963), and the variation in organism characteristics at various sites within the colony (Whittaker and Drucker 1970). This latter variation within colonies varies greatly with the type of organism, for example Streptococcus mutans has chains of cells covered in extracellular material surrounding spongy spaces in the centre of the colony, and closely packed cells without any covering at the periphery (Whittaker & Drucker 1970); conversely Bacillus cereus has oriented cells without any extracellular polysaccharide in the centre, and closely packed cells with extracellular material at the periphery. Also, Kawaguchi

(1971) reported four morphological types of cell within each of three defined areas of colonies of Mycoplasma fermentans.

The nutrient uptake by attached microbial films has been studied under batch and continuous culture conditions, but the information does not provide a clear picture. Swilley (1964) and Swilley & Atkinson (1963) defined two phases in a study of a film-flow reactor : a homogenous system in which substrate flowed through the microbial population reacting with organisms at all points in the liquid phase, and a heterogenous system in which liquid flowed over the biological film and in which reaction occurs only at the surface of the biomass. Results with glucose indicated that the diffusion rate of the substrate in the liquid phase was the limiting mechanism on uptake of organic substrates.

The flow conditions of the liquid phase are also extremely important in determining the mass transfer. Turbulent conditions can exist at the surface of the bacterial layer which permits active dispersion to aid diffusion of nutrients (Busch 1968). However, where the flow is laminar, the nutrient uptake is diffusion-limited (Gulevich 1967, 1968), 70 μm is the maximum thickness that a biological film can attain before diffusion to the inner cells is negligible (Kornegay & Andrews 1967).

There are two phases of growth of organisms in surface colonies:

1. Exponential phase. In this phase the nutrients are available at greater than growth limiting amounts, colony growth occurs at an exponential rate limited only by cell metabolism. A hemispherical colony results (Mueller-Ontjes 1973; Pirt 1967; Sanders 1967).
2. Arithmetic phase. In this phase the thickness of the colony is such that, in the centre of the colony the rate of diffusion of a growth-limiting nutrient can only maintain the metabolic rate. However, the availability of the nutrient at the outside of the colony

permits peripheral expansion of the colony. A flat colony of uniform thickness results (Mueller-Ontjes 1975; Pirt 1967; Sanders 1967).

The radial growth rate of surface colonies is reasonably constant (Palumbo et al. 1971; Pirt 1967). The colony height, viable count and volume all increase with time. However, the viable cell density is constant for each organism on each media (Palumbo et al. 1971). These observations confirm that colonies grow by the action of a peripheral growth region only.

The growth of most populations can be described by the "Logistics equation":

$$\frac{dN}{dt} = kN - \frac{K}{G} N^2$$

where N is the number of organisms, G the maximum population attainable, as controlled by environmental factors, and k is the growth rate constant. The kN term is the growth rate during the exponential phase, while $\frac{K}{G} N^2$ accounts for self-crowding effects (Brock 1966). There are many factors which will control the growth rate in any micro-environment at any time:

1. The genetic capability of the organisms; (cf. E. coli can achieve 85 generations/day, while Lactobacillus casei can attain only 38 under optimal conditions - Brock 1966).
2. Exhaustion of essential nutrients; this is a well known phenomenon in both batch cultures (Freter & Ozawa 1963), and bacterial chemostats (Novic 1955). Although a nutrient deficiency may stop cell growth, it may not eliminate all biosynthetic processes. For example, Streptococcus faecalis grown on a medium deficient in an amino acid required for protein synthesis, but not cell wall

synthesis, ceased dividing but continued to manufacture cell wall (Toennies et al. 1959).

3. Production of toxic substances; Hochberg & Folkman (1972) demonstrated the accumulation of metabolic inhibitors, both within a colony and within the supporting medium.
4. Physical crowding of organisms; this used to be considered an important factor but Brock (1966) doubts its influence.
5. The competitive ability of the organism; in all situations where an environmental factor is limiting - the ability of an organism to compete successfully with other organisms, either within or outside the colony, is extremely important in controlling the population size (Brock 1966).
6. Attachment to surfaces; the growth rate of cells attached to surfaces exceeds that of the same organisms when free in a liquid medium (Hattori 1972; Hendricks 1974).

There are two major kinds of population controls (Odum 1959):

1. Density-dependent control, where the rate of the event increases in proportion ot the increase in population (e.g. cessation of growth in the stationary phase, competitive and co-operative interactions between cells, including sexual reproduction).
2. Density-proportional events which affect a constant proportion of the population irrespective of the initial density (e.g. rate of killing by sanitizer).

Twomey (1975) observed that bacterial growth on the surfaces of milking machines was indeed colonial. However, the nature of colonial growth in food films has not been studied.

Interactions Between Organisms

Another topic that has not been studied in relation to bacteria on equipment surfaces is the interactions between organisms. The only information available are gross descriptions of the numbers and types of organisms that are present. However, many of the principles of bacterial ecology reviewed by both Brock (1966) and Alexander (1971) are applicable to bacteria on the food-contact surfaces.

An ecosystem is the "complex of organisms in a specified environment and the abiotic surroundings with which the organisms are associated". The organisms and abiotic components of any one ecosystem are unique. At any particular site there is a "community" of organisms which includes populations of individual species or distinguishable types (Alexander, 1971).

Bacteria can potentially occupy all sites on or near the earth's surface, but the organisms which do occupy any particular site are a result of environmental selection - the particular combination of physical, chemical and biological determinants which govern the community. The habitat of an organism is any "area having a degree of uniformity in those characteristics deemed to be of ecological significance". Food-contact surfaces of equipment may be considered one such habitat. There is a reciprocal relationship between a bacterium and its habitat. By selecting for populations with advantageous nutritional, physiological and morphological characteristics the environment controls the composition of the community. However, the bacteria at any site can modify the biotic and abiotic features of their environment (e.g. bacterial corrosion of metal surfaces, McDougall 1969).

The organisms within a community can be divided into the indigenous

(autochthonous) species and the invaders (allochthonous) species. The indigenous organisms are natives of the site, and will grow and contribute their metabolism to the community. The invaders are merely transient, having been transported there and are not usually capable of growth (Alexander 1971). In any site there is usually a wide diversity of species. The numbers and locations of any species within the ecosystem will be defined at any time by the controlling environment, but the patterns may change rapidly with time. Some of the organisms will dominate the ecosystem, contributing the greatest activity of the system, and therefore controlling the other organisms. A secondary group of associated organisms will be dependent on the activity of the dominants for their development, and a third group of incidental organisms may be indifferent to the activities of both dominants and associates (Brock 1966).

An ecosystem is an open system in steady state, and as such can be analysed thermodynamically (Eyring et al. 1960). Most ecosystems are extremely complex with myriad interactions between the individuals. These systems which possess tight coupling attributes have a number of integrative properties (Brock 1966):

1. Homeostasis - the ability of the system to maintain itself in the face of varying external influences over short periods;
2. Evolution - the ability to adapt to long-term changes in the environment;
3. Defence - a response to extreme external influences;
4. Repair - replacement of a lost portion of the system; and
5. Reproduction - the complete replacement of a system by one which is similar or identical (Brock 1966).

Bacterial species have evolved to permit their colonisation and

growth in a specific range of habitats. This range may be altered by selection of organisms most fit for a new environment. This selection is facilitated in bacteria by the great numbers of cells which colonise any site, compared to larger organisms. As the probability of developing a mutant is constant over all organisms, bacteria are more likely to develop mutations which will help them adapt to an environment (Brock 1966).

Fitness traits that permit an organism to cope with the environment (e.g. temperature tolerance) are the basis of natural selection. Some of the features important in interspecific competition are growth rate, physiological or cytological attributes, and protective structures. Intra-specific competition can be very important between individuals in a dominant population in a mono-specific community. This results in the development of strains, variants or mutants, which are most fit and the decline of those less fit (Alexander 1971).

Many bacteria acquire the ability to grow in environments inhospitable to the original population. These adaptive changes permit the individual or population to survive in a changed habitat. The ability of various organisms to adapt to this stage of greater fitness varies greatly. Changes of this nature may result from either phenotypic or genotypic adaptation (Alexander 1971).

In any habitat there is continual change in the composition of the community in response to modifications in the environment. The pioneering organism at any new site must possess invasiveness - the ability to migrate and establish itself in a location that possesses unexploited resources. In the absence of other organisms the pioneers have a relatively large amount of nutrients available per cell, and they may establish successfully in sub-optimal conditions. The pioneers will

grow until an environmental factor limits the population size, with usually one species dominating the pioneer community (Alexander 1971).

In bacterial successions, changes occur in both the species composition and the relative abundance of the species in the community. In these successions there is a gradual development from the pioneer community which contain few species, through a series of stages which are characterised by increasing numbers of bacterial groups, to a phase which typically has a high degree of species diversity. There are two types of succession:

1. Autogenic succession - where the resident populations alter their environment in a manner which causes them to be replaced by species more suited to the altered habitat; and
2. Allogenic succession - where one type of community is replaced by another because the habitat is altered by non-microbial factors (Alexander 1971).

Alexander (1971) has described a number of factors which determine or affect succession:

1. Provision, by one population, of a nutrient that confers an ecological advantage on the species next in the succession;
2. Provision, by one population, of a factor present in insufficient supply to allow growth of later populations;
3. Alteratian of the concentration of inorganic nutrient;
4. Modification of heterogenous substrates to expose previously physically-unavailable nutrients to succeeding organisms;
5. Autointoxication effected by existing community;
6. Bacterial elimination by physical methods;
7. Barriers associated with environmental feedback;
8. Seasonal changes in environmental conditions;

The ability of an organism to colonise a habitat and establish itself as part of a community is influenced by many factors. It must obviously be present at the site at the appropriate time and able to obtain all of its nutrients from the ecosystem. It must have the capacity to tolerate all of the ecologically significant abiotic factors in the environment and be able to grow rapidly. It should also possess mechanisms to cope with environmental resistance to colonisation due to either abiotic or biological features of the habitat. The possession of specialised structures which permit it to adhere to surfaces is obviously advantageous and, also, the possession of long-term survival capability, coupled with the ability to rapidly become metabolically active in the new environment can influence the coloniser's success (Alexander 1971).

Eventually, at any site a climax community is developed. This results from the reciprocal interactions between the bacterial and non-bacterial components of the ecosystem, which tend towards a stability in which the biotic and abiotic factors are in harmony with the environment. The climax community is not static, but is a self-replicating entity, in which the population densities of the component species are relatively stable. Despite this stability the climax may sometimes be modified by drastic exogenous influences. The climax community contains many niches, and the organisms occupying any niche are uniquely fitted to that ecosystem (Alexander 1971).

Between any two organisms in a habitat there are a number of interactions which can occur. Consequently, the number of intra- and interspecific interactions which can occur between the many organisms in any habitat are extremely complicated. The basic interactions are (Alexander 1971; Brock 1966):

1. Neutralism - no interaction between the populations. This may result from either spatial separation, or because the environmental conditions required by the organisms are so different that neither organism alters the qualities needed by the other;
2. Commensalism - one organism is benefitted and the other is not affected. This may result from;
   2.1 One population converts a substrate unavailable to a second population into a product which serves as a macronutrient for the latter,
   2.2 One species removes factors from the environment which are inhibitors to the commensal,
   2.3 One organism provides nutrients, protection or shelter to the commensal living within it.
3. Mutualism (Protoco-operation) - interaction between two species where the presence of both is required for growth in a specific environment;
4. Competition - an interaction between two organisms for a single resource, which is available in limiting amounts, and results in both organisms being disadvantaged.
5. Amensalism (antagonism) - an interaction between two organisms in which one organism is deleteriously affected;
6. Parasitism - occurs when an organism feeds on another, usually larger, organism which is injured in the process.
7. Predation - occurs when one organism feeds on and causes the death of a unicellular prey, or destruction of part of a multicellular prey.
8. Symbiosis - occurs when dissimilar species sometimes coexist intimately and exert mutually beneficial effects.

The Microflora of the Surfaces of Milking Machines.

As outlined in the previous section, many environmental factors will be influencing the numbers and composition of the flora on food-contact surfaces. Twomey (1975) demonstrated the selective effect of temperature on a coryneform-dominated flora when heat-sanitizing was routinely applied to raw milk films on stainless steel surfaces. If the heat was removed, the flora changed to one dominated by Gram negative and spore-forming organisms. Reimposition of heat removed the Gram negative bacteria and permitted the coryneforms to re-emerge. Coryneforms were particularly able to survive heating and desiccation. Myhr (1958) also found heat-sanitizing selected thermoduric organisms (micrococci and streptococci). However, the heat and other sanitizing treatments imposed did not alter the heat resistance of the organisms.

Bacteria require a physical niche ("harbourage") in order to survive on food equipment surfaces - on dairy equipment surfaces this is supplied by milkstone or corroded equipment (Daufin et al. 1970; Maxcy 1969a). The total counts in such deposits may range from 1.8 to 270 x 10^6/g (Abo-Elnaga and Kandler, 1965). Niches of this type are more available on rubber surfaces where chemical sanitizers may not penetrate (Major 1962b) and sometimes heat is ineffective (Jackson & Clegg 1965). This is reflected in the higher bacterial numbers present in rinses of milking machine clusters compared to steel parts like pails and coolers (Table 1.5-9 - Griffiths & Thomas 1959).

The bacterial counts obtained with rinses of clusters are summarised in Table 1.5-10, from the data of Palmer & Cowhig (1968) and Thomas & Thomas (1977). The interpretation of the rinse counts is (Thomas & Thomas 1977b):

1,000/m^2 - very satisfactory,

1,000 - 5,000/m^2 - satisfactory,

5,000 - 25,000/m^2 - Fairly satisfactory - could be improved,

25,000/m^2 - unsatisfactory.

The data in Table 1.5-10 show heat sanitizing (steam or boiling water) to be superior to most forms of chemical sanitizing. Of the chemical sanitizing methods, both the acidified-boiling-water system and the detergent-hypochlorite system gave excellent results, this latter system being particularly effective when complemented with periodic defatting of teat cup liners and heat treatment (Thomas 1964; Thomas et al. 1963). The QAC sanitizing system gave satisfactory results, although it was not quite as efficient as the detergent-hypochlorite system. The wet-storage system, where the machine parts were immersed in 0.3 - 0.5% NaOH between milkings, gave reasonable results only. Where 2 - 3% NaOH was used in the immersion cleaning system the results were superior to the wet-storage system, but not as good as the detergent-hypochlorite system.

The bacterial flora of rinses of milking machine clusters is presented in Table 1.5-11, based on the data of Thomas (1964), Thomas et al. (1966), Palmer & Cowhig (1968). The rinses with log colony counts had 1,000/m^2, with the high counts being 25,000/m^2. On clusters sanitized by heat or the acidified-boiling-water system, the flora was dominated by micrococci and corynebacteria, with a significant portion being heat-resistant aerobic spore-formers. Very few Gram negative rods were present.

With all of the chemical sanitizing systems the low and high counts had dissimilar flora. The flora with the low count immersion cleaning system was similar to that of heat sanitizing, with micrococci

Table 1.5-9: Comparison of bacterial numbers on rubber and steel surfaces of milking equipment (from Griffiths & Thomas 1959).

Surface	Number of rinses	Distribution of total colony counts/m^2 (%)				
		1,000	1,000-5,000	5,000-25,000	25,000-250,000	>250,000
Rubber (clusters)	1,107	22.8	11.0	12.6	23.1	40.5
Steel (milking pails, strainers, coolers etc)	1,173	36.2	13.8	16.2	19.5	14.3

Table 1.5-10: Distribution of total colony counts of rinses of milking machine clusters.

Cleaning and sanitizing system	Number of Farms	Number of Rinses	Per cent distribution of colony count ($10^2/m^2$) 10	10-50	50-250	>250	References
Steam or boiling water	6	780	77.5	15.1	5.0	2.4	Hobson et al. (1959)
	8	1,248	79.1	11.8	5.0	4.1	Thomas et al. (1963)
	20	1,348	78.7	12.3	5.0	4.0	Thomas (1964)
Detergent-sanitizer sanitizing	10	182	58.8	23.6	14.8	2.8	Hobson et al. (1959)
	20	1,020	78.0	14.3	5.4	2.3	Thomas et al. (1963)
	21	906	42	20.5	17.9	19.5	Thomas (1964)
QAC detergent-sanitizer	8	890	55.8	17.0	12.6	14.6	Thomas et al. (1963)
Wet storage of clusters	5	100	22.0	37.0	25.0	16.0	Hobson et al. (1953)
Immersion cleaning	10	780	45.3	23.9	18.3	12.5	Thomas (1964)
Acidified boiling water	10	268	61.2	19.4	15.0	3.4	Palmer & Cowhig (1968)

Table 1.5-11: Proportional distribution of rinses of milking machine clusters

Key:	Reference A	Thomas & Thomas (1977)
	B	Palmer & Cowhig (1968)
	Strep.	Streptococci
	Micro.	Micrococci
	Corynebact.	Coryneforms
	Aspor.	Asporogenous
	Gr +	Gram positive
	Gr -	Gram negative
	Coli-aerog.	Coli-aerogenes group
	Anae.	anaerogenic
	Cult.	Cultures
	Detgt.-hypo	Detergent hypochlorite

Cleaning system	Comments	Ref	Per cent distribution of organisms								No. Cult.	No. Rinse
			Strep	Micro	Cory-nebact	Other aspor. Gr + rods	Coli-aerog.	Anae. G- rods	Aerob spore-formers	Others		
Steam or hot water	low counts	A	0.9	45.6	13.2	14.0	0.8	8.6	13.8	3.1	1466	68
Imm cleaning	low counts	A	0.0	57.0	10.3	12.5	1.9	12.6	5.1	0.6	312	14
	high counts		27.0	33.0	0.0	2.0	0.0	26.0	2.0	0.0	200	10
Detgt-hypo	low counts	A	4.2	72.8	6.2	7.3	0.0	4.2	4.7	0.6	539	23
	high counts		15.5	19.7	2.7	6.1	6.9	4.3	5.9	2.9	375	16
QAC	low counts	A	8.0	32.3	1.0	3.1	0.0	43.7	10.9	1.0	192	10
	high counts											
	- pipeline		4.3	19.5	2.3	1.2	7.7	62.4	0.6	2.0	348	16
	- immersion		35.1	46.9	1.0	3.1	3.6	10.3	0.0	0.0	194	9
Not stated	Heavily contaminated	A										
	- clusters		7.2	31.5	8.9	4.4	15.6	29.4	2.1	0.9	1871	78
	- milk tubes		1.6	37.3	14.1	4.4	13.2	15.3	13.5	0.6	319	14
Acidified boiling water		B	4.7	59.8		29.6		3.5	1.2	1.2	169	100

dominating. Conversely, in the rinses with high counts, streptococci, Gram negative rods and micrococci were equally represented. Surfaces of clusters cleaned by the detergent-hypochlorite system were dominated by micrococci on low count machines and Gram negative rods on high count machines. The flora on clusters cleaned with the QAC system differed from the other chemical sanitizing systems and had some micrococci, but Gram negative rods predominated as a result of the greater resistance of these organisms to these sanitizers. Coliforms were present in significant numbers on the high count QAC-cleaned clusters. The flora of clusters and milk tubes from very badly contaminated equipment again had micrococci as dominant organisms, but with significant populations of Gram negative rods and coli-aerogenes organisms.

The distribution of total counts obtained with rinses of pipeline milking machines is summarised in Table 1.5-12 from the data of Thomas (1964-T64), Egdell & Widdas (1962-E62), Druce & Thomas (1972-D72), Thomas et al. (1966-T66), Palmer (1977, P77), and Parry & Egdell (1969-P69). Steam sanitizing method was the most efficient bacteriologically, but again some of the chemical systems are also satisfactory. The detergent-hypochlorite system gave satisfactory results only if the initial circulation temperature exceeded 82°C. The acidified-boiling-water system was also satisfactory, but it too relied on the heat to complement the chemical sanitizing action. With the lye-flooding system, the results were barely satisfactory, with one third of farmers requiring drastic improvement. The cold circulation system using a caustic-based detergent was also not very satisfactory.

The published information on the microflora of rinses of pipeline

Table 1.5-12: Distribution of total colony counts of rinses of pipeline milking machines

Key:
- T64 Thomas (1964)
- E62 Egdell & Widdas (1962)
- D72 Druce & Thomas (1972)
- T66 Thomas (1966)
- P77 Palmer (1977)
- P69 Parry & Egdell (1969)

Cleaning system	Ref	Number of		% distribution colony count $10^2/m^2$			
		Farms	Rinses	10	10 - 50	50 - 250	250
Steam sanitizing		2	662	78.7	11.5	5.6	4.2
Chlorinated-alkali-circulation cleaning:							
- survey	E62	22		59.0		21.0	20.0
- initial temp. 82°C	D72	15	245	50.2	26.1	11.0	12.7
- initial temp. 45 - 60°C	D72	12	230	20.0	12.2	23.0	44.8
- initial temp. 45°C	T64	6	161	14.3	11.8	20.3	50.9
- v. poorly cleaned	T66	21	35	0.0	0.0	0.0	100.0
- advisory rinses	D72	229	980	30.6	13.6	18.7	37.1
Caustic (lye) flooding	D72		260	24.4	21.9	20.6	33.1
Cold caustic circulation	P77		24		62		38
Acidified boiling water							
- survey	P69		291	63.6	-	21.0	15.4
- advisory rinses	D72		420	32.5	31.0	19.5	17.0

Table 1.5-13: The proportional distribution of microorganisms in rinses of pipeline milking machines

Key:		
	T63	Thomas (1963)
	D72	Druce & Thomas (1972)
	C66	Cousins et al. (1966)
	P77	Palmer (1977)
	Strep.	Streptococci
	Micro.	Micrococci
	Cory.	Coryneforms
	Gr +	Gram positive
	Gr -	Gram negative
	Aspor.	asporogenous
	Aerog	aerogenes
	Anae.	anaerogenic
	Yst	yeast

Cleaning system	Comments	Ref	Per cent distribution of organisms											No. cultures	No. rinse
			Strep.	Micro.	Cory.	Other Gr+ aspor. rods	Gr + rods	Coli-aerog.	Anae. Gr- rods	Gr- rods	Aerob. sporing rods	Streptomycetes	Yst	Other	
Detergent-hypochlorite, hot	Temp.	T63													
	60°C GpX		75	14			3			5	2			1	1300	52
	60°C GpY		9	18			18	19	28		7			1	467	18
	80°C		7	56			25			8	3			1	905	38
	GpA	D72														
	- low count		3.8	43.2	15.6	7.1		0.2	13.9		12.6	1.4		2.2	1274	57
	- high count		3.1	23.9	14.6	8.4		10.7	27.6		9.0	0.8		1.9	522	21
	Gp B															
	- low count		68.8	17.0	1.3	4.0		0.9	1.3		5.8	0.8		0.1	676	30
	- high count		55.3	22.2	6.1	0.7		0.4	13.0		1.6	0.0		0.7	455	16
Detergent-hypochlorite, cold	Hypo rinse	C66	33.5	20.0	12.9			0.0	30.0					0.7	140	7
	HNO_3 rinse		58.6	15.0	2.1			0.7	19.3					2.9	140	7
Cold caustic circulation		P77	4.2	25.0			58.3			8.3			4.2			24

milking machines is summarised in Table E-5. This is based on the data of Thomas (1963); Druce & Thomas (1972); Cousins et al. (1966) and Palmer (1971). Druce & Thomas (1972) classified the microflora of the detergent-hypochlorite cleaned machines into two groups; A - in which the flora was dominated by a combination of micrococci and streptococci regardless of total rinse count; and B - which were dominated by streptococci in both low and high count rinses. The circulation temperature had a similar effect on these pipelines as it did on the cluster surfaces, with micrococci and Gram positive rods dominant at high temperature, but either streptococci or Gram negative rods dominate at the lower temperatures (Thomas 1963).

The final sanitizing rinse in the cold detergent-hypochlorite system altered the microflora. The more acid resistant streptococci dominated the machines rinsed with nitric acid, with some micrococci and Gram negative rods. The samples from machines with the hypochlorite final-rinse still included a significant proportion of streptococci, but had considerably more rods, both Gram positive and Gram negative.

Where the cold caustic circulation cleaning system was applied to pipelines the surface flora was dominated by Gram positive rods and micrococci, with some Gram negative rods.

Summarising both cluster and pipeline microflora data it appears that:

1. Micrococci are present in significant numbers on all milking machine parts under all conditions. They are often the dominant group on low count machines suggesting they are the most resistant to removal and killing.
2. Streptococci usually occur when chemical-sanitizing, rather than heat-sanitizing is applied, and particularly when the equipment is poorly

Table 5.1-14: Classification of dominant organisms in rinses of 18 milking machines (from Druce & Thomas 1972)

Dominant types and dominant groups of bacteria in microflora of rinses	Rinses		Pipeline milking plants
	No.	%	
Streptococci	78	25.1	4
Micrococci	94	30.2	8
Corynebacteria	29	9.3	1
Other Gram +ive non-sporing rods	5	1.6	—
Coli-aerogenus organisms	8	2.6	1
Anaerogenic Gram —ive rods	37	11.9	2
Aerobic sporeforming rods	18	5.7	1
Micrococci and streptococci	19	6.1	1
Micrococci and corynebacteria	8	2.6	—
Micrococci and sporeformers	3	1.0	—
Micrococci and Gram —ive rods	3	1.0	—
Corynebacteria and Gram +ive rods	3	1.0	—
Corynebacteria and sporeformers	2	0.6	—
Corynebacteria and coliforms	3	1.0	—
Corynebacteria and Gram —ive rods	1	0.3	—
Totals	311	100.0	18

cleaned and total rinse counts are high.

3. Corynebacteria never dominate the population but are often a significant proportion of the total population when the machine is sanitized using heat, alkali or chlorine agents.
4. Gram negative rods usually only occur either on poorly cleaned chemically sanitized equipment with high rinse counts, or where a sanitizer which they tolerate is applied (e.g. QAC). The coli-aerogenes group rarely dominate the population but may be a significant proportion when the other Gram negative rods also proliferate.
5. Aerobic sporeformers can form a significant part of the flora in low count rinses when the equipment is sanitized with steam, chlorine or QAC.

The dominant microflora of 18 pipeline machines cleaned by the detergent-hypochlorite circulation system over a long period is presented in Table 1.5-14. Bacterial types which constituted more than 50% of the isolates from a rinse were considered to be the dominant type. Single bacterial species were dominant in most of the rinses (86%), and all of the remainder were dominated by distinct co-dominant groups (e.g. micrococci and corynebacteria). Nearly all of the bacterial types identified did at some stage dominate in at least one rinse. This shows the dynamic nature of the population on the milking machine surfaces. However, the organisms which dominated the machines over the long term were micrococci, thermoduric streptococci, corynebacteria, coli-aerogenes organisms, anaerogenic Gram negative rods, and aerobic sporeforming rods. Also, it must be remembered that, as the environment within a milking machine varies considerably from site

Table 1.5-15: Milk spoilage activity of isolates from rinses of farm dairy equipment (from Thomas 1974)

Cleaning and sterilisation of equipment	Per cent cultures giving various reactions in litmus milk in 72h at 22°C							
	Low count rinses; $< 1,000/m^2$				High count rinses; $< 25,000/m^2$			
	No. of cultures	Acid	Proteo-lysis	No change	No. of cultures	Acid	Proteo-lysis	No change
Steam	1,466	6.9	7.7	82.7	—	—	—	—
Immersion cleaning	312	5.7	5.1	86.5	200	8.0	7.0	77.0
Detergent-hypochlorite	530	8.0	7.7	81.7	375	29.4	23.7	38.9
QAC	192	8.9	39.1	47.8	348	24.4	41.1	30.2
Circulation cleaning*	905	5.0	4.5	87.4	1,767	63.7	7.7	19.5

*Pipeline milking plants

Table 1.5-16: Incidence of litmus milk reactions by bacteria isolated from milking machines (after Thomas et al. 1963, 1966).

Type of bacteria	Cleaning Efficiency	% Incidence of litmus milk fermentations					
		No. of cult.	Acid clot	Acid only	Alkali	Proteolysis	No change
Streptococci	good	1,140	86.5	1.3	0.2	0.4	11.6
	poor	457	81.6	9.8	0.0	0.6	8.0
Micrococci	good	1,685	7.1	2.2	0.6	6.5	83.6
	poor	1,291	4.4	10.0	0.4	14.1	71.1
Corynebacteria	good	385	3.6	3.6	0.8	9.1	82.9
	poor	392	2.3	14.0	0.0	7.9	75.8
Arthrobacteria	good	53	1.9	3.8	0.0	5.7	88.6
	poor	36	8.3	5.5	11.1	14.0	61.6
Other asporogenous Gr + rods	good	308	10.4	3.5	5.2	10.4	70.5
	poor	150	12.6	15.4	2.0	15.4	54.6
Coli-aerogenes organisms	good	609	36.0	45.3	0.7	10.0	8.0
	poor	529	63.0	24.4	0.0	4.0	8.6
Non-pigmented Gr- rods	good	660	5.9	2.1	5.6	35.6	50.8
	poor	761	10.2	4.4	1.6	45.3	38.5
Pigmented Gr- rods	good	362	10.8	1.1	5.0	48.9	34.2
	poor	558	8.4	3.2	0.0	45.4	43.0
Aerobic sporeformers	good	447	2.9	0.4	1.6	25.5	69.6
	poor	173	4.6	2.3	0.6	60.7	31.8
Streptomycetes	good	25	0.0	0.0	8.0	40.0	52.0
	poor	5	0.0	0.0	0.0	20.0	80.0
Unclassified	good	91	9.9	2.2	4.4	9.9	73.6
	poor	69	14.5	10.1	1.4	9.0	65.0

to site, so the bacterial flora are not uniformly distributed within a machine (Twomey 1975).

No direct studies have been made of the biochemical activity of the organisms on the food-contact surfaces of equipment. However, some studies of the milk-spoilage activities of these organisms have been made and, as the major nutrients are derived from milk, this can provide some background information.

This type of information has been summarised by Thomas (1964) and is presented in Table 1.5-15. This shows the relationship between the cleaning system applied and the type of microflora. The majority of organisms from efficiently cleaned machines (low count) did not ferment litmus milk in 72h, with the exception of the proteolytic organisms resulting from the use of the QAC system. The cultures from poorly cleaned (high count) surfaces were generally more active, producing acid or proteolytic reactions due to the dominance of streptococci and/or Gram negative rods, although those with immersion cleaning did not follow this general pattern. However, Palmer (1977) found on five farms that half of the organisms on the surfaces of pipeline machines cleaned by a cold caustic circulation system either reduced, or produced acid, or degraded the protein present in litmus milk.

The milk spoilage activities of groups of bacteria isolated from well-cleaned (mainly clusters, hand-washed in warm detergent-sanitizers) and poorly-cleaned milking machines are summarised in Table 1.5-16. The streptococci were among the most active organisms, mainly producing an acid clot, but a few were proteolytic. Most of the coli-aerogenes group were very active acid producers, with a few being proteolytic. Most of the Gram negative rods were actively proteolytic, with some producing acid, similar to the aerobic sporeformers from poorly-cleaned

equipment. The majority of isolates in the remainder of groups were largely inactive. With most groups of organisms the isolates from poorly cleaned equipment were more active in milk spoilage.

1.5.5 THE CONTAMINATION PROCESS

Many bacteria may deposit onto a surface of a milking machine, and some of these may become attached. A few of these may survive the cleaning system and grow on the surfaces. When the milk is brought into contact with the equipment surfaces again, some of the viable organisms may be removed from the surface into the milk effecting contamination.

The factors which are important in the contamination process are the total numbers of each type of organism on the surface, and the proportions of these that are removed from the surface into the milk. The numbers of organisms effecting contamination is the most important index of the efficiency of the hygiene practices of any food processing operation, and particularly the performance of the cleaning system. Despite this, contamination as a process has not received any direct study.

During milking with a pipeline milking machine a large volume of milk (50 - 1500 l) is in contact with the surfaces of the machine for a considerable period (0.5 - 3h) while the machine is in use. The recovery of bacteria from the surfaces of the equipment has not been determined under these conditions. A few workers have rinsed the machine subsequently with a small volume of sterile milk (Eisenreick et al. 1933; Enchev 1967; Parfitt 1936, 1940; Posthumus 1954; Richard & Auclair 1962a, b; Thomas et al. 1962; Tjotta & Solberg 1955), but these techniques may have underestimated the true contamination level due to short contact time and small recovery volume. Most of the work on bacteria on machine surfaces has been done by rinsing with a small volume of quarter strength Ringers solution (e.g. MAFF 1968), which moves even further away from the real situation by using a solution

whose recovery properties may be quite dissimilar from milk. Both swabs and direct contact plates have also been used. These are limited to sampling a particular site, while the machine is static, rather than a complete, active machine, and their recovery efficiency is again poorly related to that of the milk under practical conditions.

That the true contamination process is difficult to predict is shown by the data of Maxcy (1969b), who found that contaminants did not arise from a simple deposition/growth/recovery cycle during each use of the equipment, and that bacterial "harbourages" influenced the result. Further, Twomey and Crawley (1969b) observed that thermoduric bacteria were released from milking machine surfaces discontinuously, the day-to-day variation being ten-fold.

With one particular organism (Corynebacterium lacticum) Cousins (1972) was able to demonstrate that the routine plant rinsing method with quarter-strength Ringers solution recovered approximately 20% of those in the milk. Thus, in a machine (area $4.5m^2$) with rinse count of $1 \times 10^8/m^2$ and a recovery of 20% the total population in the milk would be 22.5×10^8. If the volume of milk passing through the machine is 45 l the increase in the bacterial count of the milk would be 5,000/ml. This establishes some relationship between rinse counts and milk contamination, but still gives no idea of the number of organisms still on the machine surfaces.

There is an incredibly large amount of information in the literature on the factors affecting the quantitative and qualitative flora of milk (e.g. Thomas 1974). The numbers of each type of organism in the milk sometimes reflect the conditions on the milking machine surface, but this is often confounded by other factors such as contamination from farm vats and road tankers and subsequent growth, cooling

treatment, age, herd characteristics etc. Consequently this information is of limited value in examining the contamination process.

1.6 SYSTEMS USED TO CLEAN MILKING MACHINES

In this section the information published since 1945 on systems used for cleaning milking machines will be discussed. The factors affecting the type of system used at any time will be examined, and the details of the various systems documented.

1.6.1 FACTORS AFFECTING THE NATURE OF THE SYSTEM USED

THE QUALITY OF MILK REQUIRED

A cleaning system has only to perform well enough to permit the milk leaving the milking machine to routinely reach whatever quality standard is required of it. In early years, the limited performance of the available cleaning chemicals, and the poor design of milking machines, made production of good quality milk a moderately difficult task. The technological advances in dairy hygiene now easily enable milk to be produced to meet the quality standards. Clegg and Cousins (1968) stated that "The standards we require for equipment bear no relation to milk standards. It is rather like building a bridge for heavy traffic and then using it only for pedestrians .....At present we demand excessively high bacteriological standards of cleanliness for equipment and then proceed to allow standards for raw milk which can only be produced on dirty equipment!". Indeed, it is so difficult to determine differences in milk quality between moderate and high performance systems, that the condition of the equipment surfaces has become the primary measure.

THE MACHINE TO BE CLEANED

The materials of which the product-contact surfaces of the milking machine are constructed play an extremely important role in determining the cleaning system applied. The characteristics of the various

materials have been discussed in Section 1.2. The improvement in the materials used has been one of the very significant advances in dairy hygiene. Stainless steel has become almost the only significant metal, which has overcome the problems of plated metals, dissimilar metals, easily-abraded surfaces and corrosion. There have also been great improvements in the types of rubber and plastic materials available. These developments in materials have removed many of the limitations on chemicals that can be used (e.g. nitric acid has come into prominence where stainless steel surfaces are used).

The change from small-volume apparatus to large-volume machines has necessitated the improvement in milking machine design. This is demonstrated by standards for machine components which specify designs which can be cleaned (e.g. 3-A standards). An example of the need to develop machine design to improve cleaning occurred when pipeline machines became popular in the UK, and attempts were made to clean them by recirculation systems. A considerable redesign of the machine was necessary before satisfactory cleaning was achieved. Alterations included spreaders in weigh jars, different jetters and alteration of the functions of the three main pipelines (Thiel 1962).

RESOURCES AVAILABLE

The resources available for cleaning also largely dictate the nature of the system applied. These resources include:

1. The chemicals available. There has been a dramatic increase in the range and complexity of the materials that are available for incorporation into dairy cleaning materials. The two most important aspects of this are the improvements in the knowledge of detergent behaviour, and development of chemical sanitizers.

2. The power available. This may be applied in terms of thermal energy (as steam or hot cleaning solutions), or as kinetic energy (as moving cleaning solutions - impinging, cascading or flowing).
3. Apparatus. The availability of apparatus designed specifically for cleaning also determines the manner in which the cleaning system is applied (e.g. pumps, dispensing apparatus, programme-controllers).
4. The available water. The amount and cost of the water available influences the volume used. Its chemical and bacteriological quality influences whether it is treated before application, and whether special detergent or sanitizer formulations are required.
5. The cleaning operator. The knowledge and motivation of the dairy man, and the cost of his labour, are of paramount importance. After a study of milking machine cleaning, McCulloch (1963) observed that where problems occurred "they were generally found to be due to either mechanical design or failure of the operator". She concluded that "further work directed towards improving the hygienic quality of milk .... must, in the main, lie in improving the general standard of care and hygiene by the operator".

PERIODIC TREATMENT

The discussion of the practice of detergency in Section 1.4.2 revealed that no cleaning treatment is totally efficient. Consequently, a soil which is resistant to a routine cleaning system accumulates. The nature and implications of this soil vary with the type of surface.

Rubberware

The accumulated soil on rubber articles is usually predominantly

fatty in composition compared to the mineral and protein soil of other surfaces. This fatty soil accelerates the deterioration of the rubber, with consequent surface degradation and loss of dimensional stability.

The frequency of the periodic treatment of rubberware is dependent on the quality of the rubber and its routine treatment. Major (1962b) found that the routine application of sodium hydroxide extended rubber life, while sodium hypochlorite reduced it. Heat sanitizing was able to kill the bacteria within the soil on the rubber, but the chemical sanitizers (iodophor, chlorhexidine and QAC) were effective on new surfaces only. Washing twice per day with a hot alkaline detergent instead of only once also extended the time between periodic treatments (Salvatierra & Murphy 1975).

Sodium hydroxide is the material recommended most widely for the periodic treatment of rubbers because it removes the fat from the rubber (Dahlberg et al. 1950; Hoy 1951; Jensen & Bortree 1946; Johns 1951; Salvatierra 1977). Iodophors can give equal performance to NaOH (Gibson & Boyko 1966) and, although Cooper (1963) found that alkaline alcohols gave cleaning superior to caustic soda, these solutions are not used commercially.

Other Surfaces

On the non-rubber surfaces of the milking machine a residual soil will accumulate. The composition and rate of deposition of the soil varies greatly with water quality, machine design and the cleaning system used (Roderig et al. 1956). Although aesthetically undesirable, milkstone is only a problem because it provides bacteria with sites protected from the cleaning solutions, and with a medium containing

ample nutrients. If heat is used for sanitizing, the organisms within the milkstone can be killed, but chemical sanitizers cannot reach the buried organisms and are therefore ineffective under those conditions (Major 1962).

There have been two approaches recommended for the problem of milkstone accumulation. The first is to periodically use heat to kill the organisms within the soil. This is temporarily effective, but the soil remains to provide nutrients and protection for the organisms deposited at the next milking. The far more effective alternative is to apply an alternative cleaning system which will effectively remove both the soil and the bacteria it contains, thus also removing the nutrients on which bacteria may subsequently grow. If an alkaline detergent has been routinely used, a common periodic treatment is the application of a high concentration of hot acid (Clegg & Cousins 1968).

PRINCIPLES OF THE DESIGN OF CLEANING SYSTEMS

One of the major factors limiting the development of cleaning systems is the lack of principles upon which a design can be based. This results from our lack of information on how the components of a system interact to influence the performance of the total system, in terms of deposit control, bacterial control, corrosion etc..

1.6.2 THE CLEANING SYSTEMS USED

There is a very large amount of information published regarding the systems applied to milking machines. In many cases the performance of the system has been well documented, but in others the information is sparse. The systems have been classified in the following way:

1. Machine design - bucket (including direct-to-can), or pipeline;

2. Heat sanitizing - where any solution within the system was applied at $\geqslant 60^{o}C$ it was classified as heat sanitized;
3. pH of main detergent - either "acidic" or "alkaline".

The details of the systems are presented in Tables 1.6-1 to 1.6-4. The information has been reduced to the first reports of systems which have performed satisfactorily on milking machines. Solutions have arbitrarily been classified as "cold" below $45^{o}C$ as "warm" between 45 and $60^{o}C$ and as "hot" $\geqslant 60^{o}C$.

PIPELINE MACHINES

Alkali-washed, Heat-sanitized

The early methods of cleaning pipeline machines (Table 1.6-1) usually involved a routine steam sanitizing, with a weekly dismantling of the machine and steaming in a chest (Thomas & Thomas 1977). This was bacteriologically efficient, producing milk with low counts (Table 1.5-12), with little milk spoilage activity (Table 1.5-14). However, the cost and high labour requirement of this method created a demand for simpler techniques, which was answered by the systems using hot-water and chemical-sanitizing. Steam was replaced, in the system of Petrova (1975), by the hot detergent sanitizer (Cl or QAC), but a weekly dismantling was still required.

The need to dismantle the machine weekly was overcome by the periodic use of acidic detergents. Nielsen et al. (1951) applied the alkaline detergent for after milking for 5 consecutive days, and the acidic detergent for the remaining two days of the week, sanitizing the machine by heat or chemicals after washing (Table 1.6.1). Whittlestone (1952) applied the acidic detergent for only one day out of seven but added a pre-milking rinse of sodium hypochlorite.

Table 1.6.1: Specifications of systems using alkaline detergents,with heat sanitizing,for cleaning pipeline milking machines.

Key:	san.	sanitizer	M	morning only
	det.	detergent	E	evening only
	alk.	alkaline	Fl.	flushed
	Cl.	chlorine	Recirc.	recirculated
	I	iodophor	Dism.	dismantled
	2X	twice per day		

System name	Reference	Components of system								Method of application	Comments
		Time of day	Pre-milk	Milk	Post-milk						
					1	2	3	4	Intercycle		
Steam	Druce & Thomas 1972	2X	-	+	Cold water	Hot alk. det. Hot alk. det.	Cold water	Steam	Drain	Fl.	Dism. weekly
Det-san.	Petrova 1975	2X	-	+	Cold water	Hot Cl-alk. or QAC det.	Cold water	-	Drain	Fl.	Dism. weekly
Alternating	Nielsen et al. 1951	2X	-	+	Cold water	Hot alk. det. 5 days Hot acid det. 2 days	Cold water	Steam, hot water, Cl or QAC	Drain	Fl.	
	Whittlestone 1952	2X	Cold Cl	+	Cold water	Hot alk. det. 6 days Hot acid det. 1 day	Hot water	-	Drain	Fl.	
Circulation - separate	Middleton et al. 1965	2X	Cold Cl	+	Cold water	Hot alk. det.	Cold water	-	Drain	Recirc. det.	Monthly clean
- combined		2X	Cold Cl	+	Cold water	Hot Cl-alk. det.	Cold water	-	Drain	Recirc. det.	Monthly clean
- simplified	Scarlett 1962	M	-	+	Cold water	Hot Cl-alk. det. (or alk. det.)	Cold water (or Cl)	-	Drain	Recirc. det.	Monthly clean
		E	-	+	Cold water	Cold Cl-alk. det. ½ conc. (or Cl)	Cold water (or Cl)	-	Drain	Recirc. det.	Monthly clean
Triple	Scott et al. 1962	2X	Cold I	+	Cold water	Hot alk. det.	Hot water	-	Drain	Fl. or recirc. det.	
	Twomey 1966a	2X	Cold I	+	Cold nonionic rinse	Hot alk. det.	Hot water	-	Drain	Fl., recirc. or RF.	
	Twomey & Crawley 1969a	2X	Cold I	+	Cold nonionic rinse	Hot alk. det.	Cold water	-	Drain	Fl., recirc. or RF.	
	Norman 1965	2X	Cold I	+	Cold water	Hot QAC det.	-	-	Drain	Fl., recirc.	Monthly dism.

Circulation cleaning using alkaline detergents and chlorine sanitizers, or combined chlorinated-alkaline detergents, was introduced to the US soon after 1950 (Alexander et al. 1953),and the UK around 1956. Four variations of this type of system are presented in Table 1.6-1. Satisfactory results were not obtained unless the detergent temperature was at least 60°C, the machine was correctly designed, and a periodic descaling was applied at least monthly (Cuthbert 1961; Egdell & Widdas 1964; Nokes & Tredinnick 1965; Snudden et al. 1961; Thiel 1962). The bacteriological efficiency of this system was satisfactory when correctly applied, giving low count rinses (Table 1.5-13) and milk of satisfactory keeping quality (Druce & Thomas 1972).

A number of variations in the design of this circulation cleaning system were examined and applied. The detergent and sanitizer could be applied either separately or combined, Middleton et al. (1965) finding little difference in the performance of the two methods, but Swift et al. (1963) finding the combined system superior. The sodium hypochlorite sanitizer gave superior bacterial control when applied as a pre-milk solution, rather than a post-wash solution (Middleton et al. 1965). Many farmers simplified the system by applying the full system once per day only,and a simplified system in the evening - an example of this type of system described by the US Milk Ordinance Code is presented in Table 1.6-1 (Cited by Scarlett 1962).

Scott et al. (1962) introduced a system which utilised the fact that iodophor sanitizers contain both surface-active agents and phosphoric acid, and can therefore function as mild acidic detergents, as well as sanitizers (Table 1.6-1). This system gave control of milkstone and surface bacteria superior to the 6 day/1day alternating system and a system using a chlorinated-alkaline detergent (Scott et al. 1962;

Whittlestone et al. 1964). Twomey (1966a) found that the incorporation of a nonionic wetting agent into the post-milking rinse improved detergency, although an anionic surfactant was not found effective in laboratory trials (Clegg & Cousins 1970). The cost of the system could be reduced by dropping the temperature of the post-detergent rinse from 96°C to 10°C without adverse affect on the milk quality (Twomey & Crawley 1969; Hubble et al. 1976). Alternatively, a hot QAC detergent-sanitizer could be substituted for the alkaline detergent and hot rinse (Normal 1965).

Alkali-washed, not Heat-sanitized

The different types of system which fall into this category have been presented in Table 1.6-2. All of these rely on chemicals for sanitizing. Some apply the detergents at warm temperature, the remainder cold. A simple system was described by Schalichev et al. (1968) in which the machines were rinsed with water and then soaked in sodium hypochlorite during the intercycle period. However, the machines required dismantling for major cleaning weekly.

The soaking of the machines in a caustic solution for the inter-cycle period was used in the "caustic-(or lye-) flooding" technique. Similar results were obtained by both Egdell and Widdas (1964), who used a pre-milking chlorine sanitizer, and O'Callaghan et al. (1965) who used water. The system achieved satisfactory bacteriological results where the machines had well-designed components, regularly-replaced rubberware and periodic dismantling and washing. With the monthly re-use of a relatively cheap detergent, there are significant savings in capital and operating costs, but not in the labour required (Egdell & Widdas 1964; O'Callaghan et al. 1965).

Table 1.6-2: Specifications of systems for cleaning pipeline milking machines, using alkaline detergents without heat sanitizing

Key:				
	san.	Sanitizer	M	morning only
	det.	detergent	E	evening only
	alk.	alkaline	Fl.	flushed
	Cl.	chlorine	Recirc.	recirculated
	I.	iodophor	Dism.	dismantled
	2X	twice per day		

System name	Reference	Components of system									Method of application	Comments
		Time of day	Pre-milk		Milk	Post-milk						
			1	2		1	2	3	4	Intercycle		
Dismantle	Shalichev et al. 1968	2X	-	-	+	Cold water	-	-	-	Cold Cl.	Fl. then soak	Dism. weekly
Caustic flooding	Egdell & Widdas 1964	2X	Cold Cl	-	+	Cold water	-	-	-	Cold caustic	Fl. then soak	Monthly re-use, periodic clean
Caustic circ. iodophor	Palmer & O'Shea 1973	2X	Cold water	-	+	Cold water	Cold caustic det.	Cold water	Cold I	Not drained	Recirc.	
Caustic circ. nitric acid	Palmer & O'Shea 1975	2X	Cold water	-	+	Cold water	Cold caustic det.	Cold water	Cold nitric acid	Not drained	Recirc.	
Caustic circ.	Palmer 1977	2X	Cold water	-	+	Cold water	Cold caustic det.	-	-	Not drained	Recirc.	Monthly clean
Chlorinated-alkali	Terplan et al 1969	2X	Cold Cl	-	+	Cold water	Warm Cl-alk. det.	Cold water	-	Drain	Fl.	
	Orr & Baines 1976	2X	-	-	+	Warm Cl-alk. det.	Warm water	-	-	Drain	Fl.	
Complementary	Fluckiger et al. 1971	M	-	-	+	Cold water	Warm Cl-alk. det.	Cold water	-	Drain	Recirc. det.	
		E	-	-	+	Cold water	Warm I det.	Cold water	-	Drain	Recirc. det.	
QAC	Armitt & Smith 1964	2X	Cold water	-	+	Cold water	Warm QAC	-	-	Drain	Fl. then recirc.	
QAC soak	Whittlestone & Murnane 1962	2X	Cold water	-	+	Cold water	-	-	-	Cold QAC	Soak	
	Feagan & Murnane 1965	2X	Cold Cl	Cold water	+	Cold water	-	-	-	Cold QAC	Soak	

A caustic-based detergent has also been used by Palmer and his co-workers for recirculation cleaning of pipelines (Table 1.6-2). In their initial system, after the detergent was rinsed away, an iodophor sanitizer was circulated and then left in the pipelines, without drainage during the intercycle period (Palmer & O'Shea 1973). Nitric acid was later successfully substituted for the iodophor (Palmer 1974). Finally, the post-detergent rinse and acidic sanitizer were eliminated, by leaving the caustic detergent undrained through the intercycle period. The quality of the milk from machines washed by this system was satisfactory, but the control of bacteria on the equipment surfaces was only moderate (Palmer 1977).

The use of a system with a warm chlorinated-alkaline detergent recirculated for 15 min. (Table 1.6-2) gave satisfactory results (Terplan et al. 1969). However, when circulation cleaning systems (Table 1.6.1) were applied at temperatures below 60°C the results were unsatisfactory (Cousins et al. 1966; Swift et al. 1963). When the chlorinated-alkaline detergent was flushed through the machine at 52.5°C immediately after the milk, the milk quality was satisfactory, although the counts of machine surfaces were poor (Orr & Baines 1976).

In the complementary system (Table 1.6.2), where an iodophor and chlorinated-alkaline solution were alternately used as the detergents, the numbers of bacteria in the milk and on machine surfaces were slightly higher than the reference technique (Fluckiger et al. 1971).

In the system described by Armitt & Smith (1964) a QAC was applied as a combined detergent-sanitizer and left unrinsed on the machine surfaces between milkings (Table 1.6-2). This system gave satisfactory total and thermoduric counts in the milk (Armitt & Smith 1964), but excessive coliform counts (Twomey 1966b). Raising the

temperature of the QAC to 88°C eliminated the coliform problem but caused the buildup of a brown deposit (Twomey 1966b).

Whittlestone and Murnane (1962) described a system in which a QAC detergent-sanitizer containing a foam-stabiliser was used to soak pipeline machines between milkings (Table 1.6-2). This system provided satisfactory cleaning with negligible corrosion. Later, the inhibition of cheese starters by residues of the QAC in the milk was detected by Feagen and Murnane (1965), a phenomenon also observed by Wildbrett when using QAC's (Wildbrett 1962). This was overcome in practice by rinsing the QAC from the machines with cold sodium hypochlorite, followed by a water rinse (Feagen & Murnane 1965). Further work with radioisotopically-labelled QAC demonstrated that the residue could also be reduced or eliminated by pre-rinsing with a nonionic surfactant, or an amphoteric sanitizer (Wright & Whittlestone 1967). Some problems were still experienced, however, with the bactericidal spectrum of the QAC (Cousins et al. 1963; Plunkett 1967 personal communication), and the system was not used commercially. A similar QAC system, without the foam-stabiliser, also gave unsatisfactory cleaning (Nezval et al. 1962).

Acid-washed, Heat-sanitized

Strong acids were first used by Pedersen and Moller-Madsen (1959), who found nitric acid prevented milkstone formation and gave satisfactory bacteriological results. Clough et al. (1965) developed the acidified-boiling-water (ABW) method (Table 1.6-3) in which the acid detergent is flushed through immediately after the milk, and sanitizing is achieved by both the hot acid detergent and following hot rinses. The system has been applied satisfactorily to pipeline machines in a

Table 1.6-4: Specifications of systems for cleaning bucket milking machines

Key:				
	San.	sanitizer	M	morning only
	det.	detergent	E	evening only
	alk.	alkaline	Br	brushed
	Cl.	chlorine	Fl	flushed
	I	iodophor		
	2X	twice per day		

Table 1.6-3: Specifications of systems for cleaning pipeline milking machines with acidic detergents applied twice daily

System		Reference	Component of system					Application method	Comments
					Post-milk				
Class	Name		Pre-milk	Milk	1	2	Intercycle		
Heat san., acid det.	Acidified-boiling-water.	Clough et al. 1965	-	+	Hot acidic det.	Hot water	Drain	Fl. or RF.	
	Acidified QAC	Heyes & Dunsmore 1978	Cold water	+	Cold nonionic rinse	Hot acid-QAC det.	Drain	Fl. or RF.	
No heat san., acid det.	Iodophor soak	Twomey 1968a	-	+	Cold nonionic rinse	-	Cold I soak	RF. then soak	
		Twomey 1968b	-	+	Cold nonionic rinse.	-	Warm I soak	RF. then soak	
	Iodophor flush	Hossack 1970	Cold I	+	Cold I/ nonionic det.	-	-	Fl.	Soft water only

Dey: det. detergent
san. sanitizer
I iodophor
Fl. flushed
RF reverse-flow

variety of conditions by both flush and reverse flow techniques (Hunter & Jeffrey 1970; Jeffrey & Hunter 1970; McKinnon et al. 1970; Palmer & O'Shea 1975; Parry & Egdell 1969). The detergents used include nitric acid, sulphamic acid and acid/surfactant mixtures (Clough et al. 1965; Palmer & O'Shea 1975). This system has achieved excellent bacteriological results in terms of both milk counts and counts on plant rinses (Table 1.5-12). It does not impair the quality of synthetic rubberware, but the life of some teat cup liners made from natural rubber may be reduced (Buck & Parry 1974).

A second hot-acid system, in which an acidic QAC is used as the detergent, achieved very satisfactory results in terms of bacterial counts in milk and on machine surfaces (Heyes & Dunsmore 1978). A small amount of QAC residues entered the milk, but was insignficant in terms of effect on the activity of cheese starter cultures (Dunsmore et al. 1978).

Acid-washed, Not Heat-sanitized

Three cold-acid systems have been described which use an iodophor as the washing solution. In the first, the machine was rinsed by a non-ionic solution applied by reverse flush, and then soaked with a cold iodophor for the intercycle period (Twomey 1968a). The temperature of the iodophor was later increased to 50°C to improve detergency (Twomey 1968b). Hossack (1977) described a system where an iodophor was applied in soft water both as a pre-milking rinse, and a post-milking wash. No performance data are available on any of these cold-acid systems.

BUCKET MACHINES

Alkali-washed, Heat-sanitized

The brush-washing of buckets and apparatus in an alkaline detergent, with subsequent sanitizing with steam or boiling water,is the traditional method of cleaning this type of machine (Clegg & Cousins 1968). This method gave very satisfactory bacteriological control (Table 1.5-10) and milk with good keeping quality (Table 1.5-11). Like the pipeline machines, the cost of and time required by this system prompted the adoption of alternative systems.

Acid-washed, Heat-sanitized

Palmer & Cowhig (1968) adqpted the acidified-boiling-water system, which is normally applied to pipeline machines, for bucket apparatus with satisfactory results. Citric acid was shown to be a suitable replacement for the nitric or sulphamic acids usually applied. However, there are problems for the operator when brush-washing with the boiling acidic detergent.

Alkaline-washing, No heat

The fact that a large proportion of the product-contact surface in bucket machines is rubber, and that caustic solutions can give both good detergency and satisfactory sanitizing, has made caustic materials the detergent of choice in many bucket cleaning systems Johns (1933) used this as the sole detergent-sanitizer in the system and merely drained it before the next milking (Table 1.6-4). Sodium metasilicate could be satisfactorily substituted for the sodium hydroxide for aliminium equipment. Dahlberg et al. (1950) used an alkaline detergent prior to the wet-storage period and removed the caustic by a rinse of sodium hypochlorite. In the UK a chlorinated-

Table 1.6-4: Specifications of systems for cleaning bucket milking machines

Key:				
	San.	sanitizer	M	morning only
	det.	detergent	E	evening only
	alk.	alkaline	Br	brushed
	Cl.	chlorine	Fl	flushed
	I	iodophor		
	2X	twice per day		

System		Reference	Component of system									Method of application	Comments
Class	Name		Time of Day	Pre-milk 1	Pre-milk 2	Milk	Post-milk 1	Post-milk 2	Post-milk 3	Post-milk 4	Intercycle		
Heat san., alk. det.	Steamed	Druce & Thomas 1972	2X	-	-	+	Cold water	Warm alk. det.	Cold water	Steam or hot water	Drain	Det. br.	Periodic acid
Heat san., acid det.	Acidified-boiling-water	Palmer & Cowhig 1968	2X	-	-	+	Hot acid det.	-	-	-	Drain	Det. br.	Periodic cl-alk. det.
No heat san., Alk. det.	Wet storage	Johns 1933	2X	-	-	+	Cold water	-	-	-	Cold caustic det.	Fl. then soak	
	- caustic	Dahlberg et al. 1950	2X	Cold cl.	-		Cold water	Warm alk. det.	-	-	Cold caustic det.	Fl. then soak	Periodic acid
	- QAC	Mallmann et al. 1946	2X	Cold QAC	-	+	Cold water	-	-	-	Cold QAC det.	Fl, then soak	
		Dahlberg et al. 1950	2X	Cold Cl	-	+	Cold water	Warm alk. det.	-	-	Cold QAC det.	Fl. then soak	Periodic acid
	Immersion - caustic	Thiel et al. 1955	2X	Cold Cl	-	+	Cold water	-	-	-	Cold caustic det.	Fl. then soak	Monthly re-use and periodic clean
	- QAC	Thiel 1958	2X	Cold Cl	-	+	Cold water	-	-	-	Cold QAC	Fl. then soak	
	Alkali, brushed	Thome & Leesement 1959a	2X	-	-	+	Cold water	Warm alk. det.	Cold water	-	Cold Cl.	Det. br.	
		Clegg & Bacic 1968	2X	Cold Cl	-	+	Cold water	Warm alk. det.	Cold water	-	Drain	Det. br.	
	Chlorinated-alkali	Palmer, 1976	2X		-	+	Cold water	Warm Cl-alk. det.	Cold water	-	Drain	Det. br.	Periodic acid
	QAC	Dahlberg et al. 1950	2X	Cold cl.	Cold water	+	Cold water	Warm QAC det.	-	-	Drain	Det. br.	Periodic acid
		Botwright 1952	2X	Cold water	-	+	Cold water	Warm QAC det.	-	-	Drain	Det. br.	
	Complement-ary	Fluckiger et al. 1971	M	-	-	+	Cold water	Warm Cl-alk. det.	Cold water	-	Drain	Det. br.	
			E	-	-	+	Cold water	Warm I det.	Cold water	-	Drain	Det. br.	
No heat san., acid det.	Iodophor-brush	Palmer 1976	2X	-	-	+	Cold water	Warm I det.	Cold water	-	Drain	Det. br.	Periodic cl-alk. det.

alkaline detergent was substituted for the caustic solution to overcome the inherent corrosion problems (Hoy & Rowlands 1948). The performance of this latter system was only moderate, but could be improved by effective cleaning of the clusters in chlorinated-detergent before the immersion period (Hobson & Thomas 1955). In the late 1940's QAC's became available and were used as an alternative to the caustic wet storage solution (Table 1.6-4). Mallmann et al. (1946) used the QAC alone, but Dahlberg et al. (1950) recommended that a detergent wash should precede the QAC storage period.

The immersion cleaning system was developed by Thiel et al. (1955) for direct-to-can equipment. It applied the principle of the wet storage method to this equipment, initially using a sodium hydroxide solution, and later incorporating EDTA (Thiel et al. 1956). Subsequently, a QAC was offered as an alternative immersion solution (Thiel 1958).

The application of the technique was later extended to include bucket machines (Whitehouse & Clegg 1961). A number of techniques necessary for its successful application were discussed by Egdell et al. (1958), Scarlett (1962) and Thiel (1958). Although the performance of the immersion technique was only moderate (Table 1.5-10) it was adequate under practical farm conditions (Clegg & Cousins 1968; Cowhig & Mulcahy 1965).

Thome & Leesment (1959a) described a system where an alkaline detergent was used for brush-washing, and the apparatus was stored in a hypochlorite solution during the intercycle period. Palmer (1976) and Richard & Auclair (1962b) recommended a combined chlorinated-alkaline detergent instead of the separate detergent and sanitizer. A complementary system, in which the chlorinated-alkaline detergent was alternated with an iodophor washing solution, was found to give

satisfactory results (Fluckiger et al. 1971).

Brush-washing systems using QAC detergent-sanitizers have been described by Botwright (1952); Dahlberg et al. (1950) and Elliker et al. (1950).

Acidic-wash, No Heat-sanitizing

A brush-washing system using an iodophor as the washing solution was described by Palmer (1976). This is complemented by periodic treatment with a chlorinated-alkaline detergent. No data is available on its performance.

1.7 ASSESSING THE PERFORMANCE OF CLEANING SYSTEMS

1.7.1 THE FACTORS TO BE ASSESSED

The function of the cleaning system is to maintain food-handling equipment in a condition which will permit the food to meet any required quality standards. The "best" system for any situation is therefore that system which meets these standards at the least cost. Thus, the overall performance of a system must be measured economically. However, the performance may also be measured indirectly by assessing either the quality of the product, or the condition of the equipment surfaces.

ECONOMIC ASSESSMENT OF CLEANING PERFORMANCE

The costs of cleaning include the following:

1. Capital costs of cleaning apparatus;
2. Operating costs in supplying water, detergents, sanitizers and labour;
3. Replacement costs of corroded or damaged equipment;
4. Costs of monitoring cleaning efficiency;
5. Costs of disposing of cleaning effluent; and
6. Costs of below-standard product when cleaning is unsatisfactory.

Unfortunately, no economic analyses have been published viewing cleaning from this wide perspective. However, there have been small studies, using partial budgets, to compare the costs of various milking machine cleaning systems (e.g. Akam 1955; Griffith-Jones 1961; Richard 1976; Thiel 1962). These are very valuable, but there are only a few compared to the large number of studies using milk quality or conditions of the machine surfaces as measurements of performance.

ASSESSING THE QUALITY OF THE MILK

Good quality milk is a concept having many attributes, all of which are important if the milk is to be a satisfactory food for human consumption.

Physical Quality

The major physical properties of milk which are routinely assessed are those of; added water content (to detect dilution) and sediment content (to prevent contamination with hair, faeces or any other particulate or coloured material). Standard methods have been described for both of these determinations (e.g. Hausler 1972).

Chemical Quality

The chemical composition and activity of milk has been discussed in many texts. One of the more recent reviews is that of Webb et al. (1974).

The chemical quality of milk is usually considered in two parts. The gross chemical composition of the major milk constituents of fat, protein, lactose and minerals are determined routinely in many milk quality assessment programmes. They may be measured by either manual or automated techniques - e.g. Babcock test or Milkotester for fat determination (Hausler 1972).

The minor components of milk include the trace elements Al, As, Ba, B, Br, Cd, Cr, Co, Fl, I, Pb, Li, Mn, Mo, Ni, Ru, Se, Si, Ag, Sr, Ti, Va and Zn; a wide range of enzymes; non-protein nitrogen compounds such as ammonia, urea and uric acid; and also flavour components, phospholipids, sterols, pigments and several vitamins. The concentration of these compounds may vary with the breed, age, stage of lactation, time of milking, time within milking, environment, reproductive

condition, nutrition and disease condition of the cow.

The chemical quality of the milk can be influenced by the presence of residues. The residues of particular interest in milking hygiene are those derived from the hygienic practices of milking or milk transport and storage on the farm. Detergents are usually followed in the cleaning system by a rinse of water or sanitizer, which prevents the detergent from being a significant source of residues in milk. However, the sanitizer or detergent-sanitizer is usually applied as the final pre-milk step of the cleaning system. This creates an opportunity for residues of these cleaning materials to enter the milk; particularly as milking machines are usually insufficiently drained (Dunsmore et al. 1977b). The influence of iodophors, QAC's and chlorine compounds on milk harvested through bucket and pipeline machines has been examined (Dunsmore et al. 1977c, 1978; Mull & Fouts 1947; Posthumus 1968; Seelemann & Wegener 1956; Swartling 1959).

Biological Quality

The biological quality of milk can be considered in two sections. Most emphasis is placed on the microbiological content of the milk, but there is also interest in the somatic cell content. The microflora of milk may include bacteria, and less frequently, fungi, viruses and rickettsia. These organisms may cause a reduction of milk quality through their biochemical activities, which may result in the degradation of important milk constituents or the production of undesirable odours, flavours or milk processing characteristics.

As one of the major tasks of cleaning is to ensure that a satisfactory bacteriological quality of the product is achieved, it has been argued that the bacteriological examination of the product itself

is the most appropriate way of assessing cleaning efficiency (Sing et al. 1967). Hartley et al. (1968) have reviewed the use of bacteriological tests of raw milk quality as indicators of the cleanliness of production conditions and Baker (1967) has since compared a number of tests. The major problem with assessment of the quality of the final product is that often no warning of an impending unsatisfactory situation is observed, and the product is spoilt before remedial action can be taken, although Sing et al. (1967) described a device to facilitate the in-line sampling of liquid products during manufacture, which may improve the control achieved in the factory situation. Cranston (1973) described a technique of using statistical examination of microbial analyses of in-line-samples as a quality control procedure. The observation that the first product over the equipment surface picks up most of the contaminating organisms, has led to the first product being used as a defined technique for both milking utensils (Jones-Evans 1948), milking machines (Burgkart-Schnepf & Camphausen 1961) and milk fillers (Wiesner et al. 1971).

Bacteriological examination of the product has been used as either, the sole measure of cleaning efficiency (Baseby et al. 1965; Thiel et al. 1955; Twomey 1966b), or in order to complement direct assessment of the bacterial numbers on equipment surfaces (Carriera et al. 1955; Clegg & Bacic 1968; Palmer & O'Shea 1973). The bacteriological analyses performed on the product samples fall into three categories:

1. Some use the tests which are routinely used to assess milk quality. The most common measurement is the standard plate count, an estimate of viable bacteria, but many programmes include assessment of other important groups such as coliforms, thermodurics and psychro-

trophs. Some organisms, such as Escherichia coli, are also routinely assessed as indices of the possible presence of pathogenic organisms (Foster et al. 1958). The bacterial content of milk may be determined directly or indirectly. The main direct methods used are the microscopic count and the plate count (Häusler 1972; Ministry of Agriculture, Fisheries & Food 1968). As these are both laborious and expensive to perform, many indirect techniques have been developed. These include the resazurin and methylene blue reduction tests, which measure the microbial activity of milk, as indicated by a change in redox potential. More recently, the Coulter Counter has been used to count colonies at an early stage of development, permitting a more rapid result to be obtained (Tolle et al. 1966). These routine bacteriological tests have been used in many studies of milking machine cleaning (e.g. Baseby et al. 1965; Carriera et al. 1955).

2. Other studies have been made on the effect of various hygienic practices upon the microflora of the milk. Much of the British work has recently been reviewed by Thomas & Thomas (1977). Some studies have also been performed in New Zealand (Twomey 1966b; Twomey & Crawley 1969a).

3. Some workers have used methods specifically designed for the evaluation of hygiene standards. Elliker (1967) advocated a 5-day plate test for processing equipment, Nikhinson et al. (1970) the Phage Growth Reaction and Taylor (1971) demonstrated the use of the water-agar test to examine milk samples taken from pasteurisers. Blankenagel et al. (1967) proposed the routine use of the enterococcus count for evaluation of creamery sanitation. Maxcy (1969b) developed a surface plate method for rapid assessment of the post-

pasteurisation contamination likely to impair product quality. Quality assessment schemes based on a number of indices, as a means of comparing operating efficiency of factories, have been described for the manufacture of cheese (Dommet 1966), pasteurised products (Contreras et al. 1970) and all products (Sauts & Tipikina 1974).

Organoleptic Quality

Milk possesses a delicate flavour which is pleasing to most people, but can easily be affected by poor treatment of the milk or addition of flavoured compounds. The exact nature of the milk flavour has not been determined, but the milkfat, protein, lactose, carbonyl compounds, methyl sulphide and somatic cells can all contribute to its characteristic flavour (Webb et al. 1974).

Technological Quality

The technological quality of milk is its ability to be used to prepare satisfactory dairy products. Techniques such as the methylene blue reduction test described above are used as an index of the keeping quality of the milk when pasteurised for the consumer. Where the milk is being used for the manufacture of fermented products, such as cheese and yoghurt, it must not contain compounds (e.g. antibiotics, sanitizers or somatic cells) which might inhibit the ability of the starter bacteria to produce acid. This tendency can be detected by an inhibition test (Ministry of Agriculture, Fisheries & Food 1968).

Toxicological Quality

The way in which milk is produced, harvested, transported, stored and processed provides many opportunities for the contamination by materials which may be toxic. The major sources of this contamination

are the diet of the cow and cow medication (e.g. antibiotics). The method of cleaning and sanitizing milk harvesting equipment on the farm can also result in detergent or sanitizer constituents entering the milk in concentrations which may be toxicologically significant (e.g. iodine from iodophor sanitizers - Dunsmore & Wheeler 1977)

ASSESSING THE CONDITION OF THE SURFACES OF THE MILKING MACHINE

The chemical and bacteriological composition of the milk delivered to the dairy factory, as described above, is influenced by the performance of the cleaning system. Unfortunately, the milk is often contaminated before entering the milking machine, or by subsequent storage and transport facilities. Also, variations in cleaning performance cause only very small changes in the numbers of bacteria in the milk until the cleaning system under examination performs quite poorly (Clegg & Cousins 1968). For these reasons the milk quality is usually regarded as an insensitive index of milking machine hygiene.

A dairy cleaning system is used to minimise the development of product residues (milkstone) on equipment surfaces and to prevent the proliferation of bacteria in these residues. Many workers have taken the attitude that the amount of product residue is an important feature in itself, because it may be an endex of potential bacterial contamination. Others believe that the level of bacterial development on the equipment surfaces is the most important criteria of cleaning performance. These different views are reflected in the variety of techniques that have been used to assess cleaning in the practical industry situation, many attempting to determine the amount of physical deposit and others the bacteriological situation.

Physical Assessment Techniques

In general, the physical assessment techniques measure the efficiency of detergency. The techniques can be divided into those which may be applied by industry personnel without complicated laboratory apparatus, and those requiring such equipment. The physical techniques which can be applied in the industry situation include:

1. Visual inspection - which has been the first, and in many cases, the only means of soil estimation, particularly in the practical situation. It is reasonably satisfactory for glass equipment, but poor for metal surfaces (Davis 1956b). Unfortunately, the accuracy of results obtained is affected by the acuity of vision of the observer, intensity of illumination, dryness of surfaces and nature of the deposit (Abele 1965). Visual inspection methods have been improved in many ways. Illumination by ultra-violet light assists the detection of organic soils, as many components of these soils fluoresce when illuminated (Harris 1954). Ultra-violet illumination permitted reproduceable and partly quantifiable assessments of physical soil in studies of vat washing (Scott et al. 1975). This technique has been further developed by the application of liquid or powdered fluorescent dyes which adhere to all types of contamination (Harris 1954). A number of techniques which use staining of the soil and inspection under natural light, have also been developed; Stack (1952) found carbol fuchsin useful for this purpose. Lindahl et al. (1966) examined the cleaning of road transport tankers, using a soil which contained fluorescein to improve sensitivity.
2. Manual tests - which consist of drawing a suitable object (such as a thumb nail or spatula) over the surface of the equipment

(Abele 1965; Davis 1956b). This crude but useful test may be further improved by the use of a white paper or glove as the wiping implement.

3. The water-beaker test (Harris 1954), where ability of water to completely wet a surface is determined, has long been popular with detergent laboratories. However, under certain conditions, water break is not a satisfactory indicator of complete cleanliness and more complicated techniques have been developed (Linford & Saubestre 1951; Spring et al. 1946).
4. A powder-dusting method (Brookes & Fennel 1952; Ministry of Food 1951), which depends on the fact that traces of grease on a utensil will retain particles of a powder applied by a blower. Armbruster and Ridenour (1952) described a procedure using talc containing the powdered dye "Safranin-O" which clung tenaciously to residual organic matter giving a red spotted appearance, and Beck (1962) found this to be a valuable test for detecting residues on glass surfaces in the practical situation.

A tremendous variety of physical assessment techniques have been used which require complicated laboratory apparatus. This limits their use to research or developmental applications:

1. Photometric examination of cleaning solutions to determine the concentration of dissolved product residues. Fischer et al. (1969) found this to be less sensitive than analysis of calcium in soil by atomic absorption spectrophotometers. However, photometers have an advantage, in that they are simple to instal in practical situations (Fliege 1970). Patel and Jordon (1964) examined the factors controlling the efficiency of rinsing by measurement of turbidity, and Ruiz et al. (1975a, b) found that turbidometric evaluation could be

successfully applied to the continuous monitoring of factory cleaning.

2. Photometric assessment of solid soils on glass surfaces. This technique has been used widely in practical and laboratory situations to assess the soil on the test surface before and after washing (Leesment 1960; Mann & Ruchhoft 1946; Norris & Ruchhoft 1948). The methods are relatively simple, but the use of a glass substrate may limit the application of the results to the cleaning of other types of equipment surface. Dunsmore (1975) used this as the basis of a laboratory technique for evaluating general purpose dairy detergents. The technique is admirably suited for the evaluation of milk bottle cleaning where the prior staining of soil with fuchsin improved sensitivity (Frisen et al. 1964).
3. Measurement of indices of surface activity. For simple systems (e.g. the removal of an oil soil), the measurement of contact angles, interfacial tensions, and similar properties may be of some value (Jennings 1965). However, contamination of the system with polar impurities may adversely affect the properties being measured (Durham 1961).
4. Gravimetric estimation of total soil deposit. This is the most direct technique, but lacks great sensitivity, as the weight change of the soil is small compared to the mass of the substrate. It can also be time-consuming, because of the need for prior drying of the sample to constant weight. However, this technique has been widely used for both bench and simulator techniques (Clegg & Cousins 1970; Hankinson & Carver 1968; Murphy 1973; Peters & Calbert 1960; Scott et al. 1962).
5. Measurement of refractive index. Fleischer et al. (1972) evaluated

detergency by measureing the ratio of refractive indices of a transparent substrate and an inorganic suspension.

6. Measurement of electrical conductance of a cleaning solution. Fisher and his co-workers (Fischer et al. 1974; Ruiz et al. 1972) found that measurement of conductance could be used to effectively monitor the rinsing operation, but variation in the interaction between different detergents and milk precluded its use for monitoring detergent solutions.
7. Measurement of electrical resistance of the cleaning solution. Fischer et al. (1969) found that the resistance of a rinse solution could be used to accurately determine the point of complete removal of ionizable soil in cleaned-in-place(CIP) system.
8. Measurement of adhered soil deposits using radioisotopes. The use of radioisotope labelling has been reviewed by Jennings (1961) and Jordon (1966). Radioisotopes offer advantages for research purposes where great sensitivity is required, being at least 100 times as sensitive as any other method (Harris 1954). A popular method is the blending of a source of radioactivity into a synthetic or natural soil. An alternative method is that in which the radiochemical forms an integral part of the soil molecule. These methods are quite satisfactory for most soils, but, for some heterogenous soil systems, the soil fraction bearing the radioactivity is not distributed uniformly through the deposit. In these cases, the decrease in radioactivity does not parallel the decrease in soil load (Jennings 1965) and erroneous interpretations may be made. An alternative method of labelling is to use bacteria previously grown on radioactive substrates and thus themselves labelled (Hays et al. 1958, 1960; Masurovsky & Jordan 1958; Peters & Calbert

1960). Incorporation of these organisms into soils allows measurement of the removal of one species of particulate soil, but again assumes that all soil is removed at the same rate (Jennings 1965). The major isotopes used have been ^{32}P, ^{45}Ca, ^{131}I and ^{198}Au, with a double-label technique being employed by Gordon et al. (1966). The techniques have been used to evaluate tank washing (Beer et al. 1966), to compare equipment surfaces, to determine the dissemination of contaminating bacteria (Vrigazov et al. 1973), in laboratory studies on cleaning (Pflug et al. 1961) and also simulator studies (Jennings 1961; Nisbet & Langdon 1975; Peters & Calbert 1960).

One side-effect of the action of the detergents and sanitizers used for cleaning can be the corrosion of the equipment surfaces. The incidence of corrosion and methods of evaluation have been reviewed (International Dairy Federation 1971). Accelerated laboratory corrosion tests, both manual and automated, have been developed (Daufin et al. 1970; Whittlestone 1963; Wildbrett 1967). Corrosion assessments have also been made in association with soil removal efficiency in some simulator techniques (Whittlestone et al. 1963; Murphy 1973).

Chemical Assessment Techniques

Chemical methods are not used in the dairy industry to routinely assess the hygienic condition of equipment surfaces. The only method used is that described by Davis (1956a), who utilised the loss of chlorine from a sanitizer solution, resulting from absorption of this chlorine to a milk soil, as a measure of the amount of soil on the surface.

In the research and development situations chemical analyses of some single components of dairy product residues are relatively simple

to perform and gave an indication of the total residue if the composition of the residue remains constant. Nitrogen analyses of soil residues were performed in studies of protein dissolution by chlorine in alkaline detergents (MacGregor et al. 1954; Merrill et al. 1961; Wright et al. 1967), and in investigations into the cleaning of equipment soiled with hot milk (Burton 1961). Calcium analysis by atomic absorption spectrophotometry of soils dissolved from equipment surfaces by HCl was described by Heinz et al. (1967). Anderson et al. (1972a) used this technique to study the cleaning efficiency of a range of acidic detergents. Later, Anderson et al. (1973a) found that detergent components only caused minor interference with the accuracy of the analysis and the technique may also be satisfactorily applied to the pipeline detection of milk deposits (Anderson et al. 1974). The technique has been used to investigate detergency at low temperatures (Polowniak et al.1969), to examine some relationships in rinsing (Anderson et al. 1972b), and to evaluate acidified-boiling-water cleaning (White & Rabe 1970).

Multiple analyses give a more accurate indication of the influence of the cleaning situation on both the amount and the composition of the product residue. Clegg and Cousins (1970) performed gross analyses on soils for "fat", "protein" and "minerals". Arnold and Maxcy (1970) have applied more rigorous techniques to the analysis of the residue from pasteuriser washing, using centrifugal fractionation, solvent extraction, gas liquid chromatography, thin layer chromatography and infra-red spectroscopy to determine the chemical nature of the more adherent components of the residue. The same techniques have been used to examine the re-use of detergents (Kulkarni et al. 1974).

Chemical analyses of detergents and sanitizers to predict their practical performance are being used less frequently as satisfactory laboratory performance evaluating techniques become available. Nevertheless Thom (1966, 1971) has described analyses for the phosphorus and silicate contents of detergents. Schlussler et al. (1970) described a technique for determining the chemical oxygen demand of liquid detergents for bottle-washing baths.

Bacteriological Assessment Techniques

The assessment of the bacteriological condition of equipment surfaces is an extremely popular method of assessing the hygienic condition of equipment used in the dairy industry. This results from the emphasis placed by health authorities on the bacteriological condition of the milk. Although these procedures are popular, Jennings (1965) claimed that they only gave an indication of the number of viable bacteria on the surface at the time of testing, but no indication of either the potential amount of bacterial growth that could be expected, or the physical cleanliness of the surface.

The four main techniques used to sample the organisms from the equipment surfaces are: rinsing, swabbing, direct application of a growth medium to the equipment surface with subsequent incubation of the equipment, and application of the surface of a solid growth medium to the equipment surface, with a subsequent incubation of the medium. These techniques have been used for assessing the efficiency of cleaning of a wide range of items including both farm and factory equipment; the rinse and swab methods being the most popular. The relative value of each of these techniques has been assessed by Walter

(1955) who reported the proceedings of a symposium on surface-sampling methods, Angelotti et al. (1958) and Niskanen & Pohja (1977) who compared a number of evaluation techniques and Baldock (1974) who reviewed some of the information on assessment of bacterial contamination of surfaces.

Surface-rinsing has been widely used for assessing the hygienic condition of enclosed equipment. The major problem with this technique is determining how efficiently it has removed the bacteria from the equipment surface. Bacic and Clegg (1967) showed by serial rinsing that the first rinse removed only approximately 10% of the available bacteria. This compares with the 10-80% reported earlier by Richard & Auclair (1962a) and Thome & Leesment (1959a), where new rubberware, which can be more easily rinsed, was installed on the machines. A rinse-filter method, using membrane filters to sterilize the rinse-water and aid in enumeration of the organisms has been described (Scroggins & Marshall 1976).

The method of rinsing greatly affects the efficiency of the operation. Milk was found to be a superior rinsing solution to either water or other aqueous solutions by some workers (Richard & Auclair 1962a; Eisenrèich et al. 1953), but not by Holager (1951). Application of the rinse at a lukewarm temperature (30 - 39°C) was found by Holager (1951) to improve efficiency, compared to a low temperature (5 - 8°C), although no significant difference was observed by Bacic & Clegg (1967). Thomas et al. (1962) demonstrated the importance of correct handling of rinse samples. The use of some means of physically agitating the solution also increased the efficiency with which it recovers organisms, glass beads in rinsing milk cans and others (Daufin & Sainclivier 1967; Favero et al. 1968) using ultrasonics.

Pulsation of the teat cup inflations while rinsing was shown by Claydon (1953) to improve the recovery of bacteria when evaluating milking equipment. Spray application of rinsing solutions has been widely used for assessment of contamination on milk tank surfaces (McKinnon & Cousins 1969; Orr & Baines 1968; Scott et al. 1975; Twomey & Crawley 1969a). Twomey & Crawley (1968) found that the cleaning systems applied influenced the manner in which bacteria were recovered by serial rinsing solutions.

Davis (1956b) reviewed the swab-contact method as a surface sampling technique for bacteriological evaluation to 1956. Baldock (1974) has reviewed the developments in swabbing since Davis' report, including comparison of materials used in the swab, swabbing in conjunction with membrane filtration, and control over swabbing technique. Swabbing continues to be popular for assessment of the bacterial condition of awkward or very large items of equipment. Thomas et al. (1952) showed that approximately 30% of the organisms on a surface were recovered by a single swabbing, with a second swabbing removing a further 30%. The keeping quality of swab samples was also shown to be inversely related to the initial bacterial content (Thomas et al. 1962). Ulbrich and Hunter (1964) examined the value of swabbing using both the Koch plate and direct smear techniques, and Leesment (1959) described a simplified swab test, to be used as a routine quality control procedure in dairy processing. Swabbing large areas has been achieved by replacing the swab by a plastic sponge (Silliker & Gabis 1975). Heiberg (1965) reported the results of considerable swab sampling of equipment in Norwegian dairies. Johns & McClure (1961) compared the use of a pulsating rinse and swabbing as a means of sampling the bacterial contamination from milking

machine surfaces and concluded that the swab test could give a seriously misleading estimation of the situation. The recovery of soil bacteria by rinsing was significantly greater than swabbing, except where the equipment was fairly clean and rubberware new (Enchev 1967; Johns & McClure 1961). O'Callaghan & Clegg (1964) observed that rinse counts were generally 1000 times greater than swab counts.

In the medium-contact techniques, a solid medium is brought into contact with the surface to be sampled and then incubated and the organisms enumerated. Baldock (1974) in his review classified these techniques into three categories; the agar slice method; impression methods using acetate adhesive tape, a plastic peelable film or membrane filter; and the direct surface agar plate. Darlan et al. (1973) described an alternative to the direct surface contact-plate in which the culture medium is coated onto a microscope slide for taking the surface sample, and the monitoring of food hygiene using these slides has been discussed by Mossel et al. (1976). Baldock (1974) compared the features of these contact methods with both swabbing and rinsing. These medium-contact techniques have become popular in the dairy industry for routine evaluation of the bacteria from the surfaces of smooth dry equipment (Koppel 1966; O'Connor 1970; Tarrant 1973).

The bacteriological evaluations performed on the samples taken from the surfaces include both the routine quality evaluations that apply locally to the milk, and examination of some features of the bacterial flora. The "coliform titre" was used to evaluate rinses and milk samples by Alagezyan (1967) and Kazarova (1969), but Kazarova (1970) and Danilenko (1971a, b) found the "enterococci titre" gave

more sensitive assessment of cleaning efficiency.

Most of the techniques described above can be applied in the industry situation. One technique that can only be applied under research conditions is that using spores of B. stearothermophilus in yoghurt culture as a means of assessing the efficiency of cleaning of milk tanks (Galesloot et al. 1967).

1.7.2 FIELD ASSESSMENT OF CLEANING PERFORMANCE

The ultimate test of any cleaning system is its performance in the practical, industry situation. Indeed, Davis (1956a) stated that "no laboratory tests can take the place of trials under practical conditions". Harris (1954) placed this in perspective by stating that the phenomenon of cleaning is so complex that "it is natural that some investigators may simply decide that the only test is a practical one, under the conditions encountered on the job. There are those too, who verbally express this attitude, but who, nevertheless, run the controlled laboratory test because it is the simplest method for screening large numbers of samples available only in small quantity. Certainly the most effective method, and the most reasonable one, is to perform the laboratory screening tests, meanwhile building up a fund of data to be correlated with practice, progressing as soon as feasible to a semi-or full-scale field trial under practical conditions of operation".

ADVANTAGES OF THE FIELD TRIAL

The field trial has two major advantages as an experimental technique:

1. The experiment is conducted under actual working conditions of water quality, soil, equipment design, apparatus for solution

application, and personnel. All of these factors will interact to influence the performance of the cleaning system in ways which often cannot be duplicated in laboratory experiments (Davis 1956a).

2. The experiment can be assessed by all of the measurements of performance discussed above, including:
   (a) the economics of the total operation,
   (b) the quality of the end product,and
   (c) the condition of the equipment surfaces.

DISADVANTAGES OF THE FIELD TRIAL

1. The conditions in the industry, in terms of water quality, soil, equipment design, cleaning apparatus and personnel, vary greatly from site to site within the industry. Unless specific measures are taken to include a wide range of conditions, the results will probably be applicable only to a specific range of practical conditions. The variation in results from different sites necessitates considerable replication.
2. Field trials must be performed over sufficient time to allow the expression of significant differences between treatments where they exist. This may range from weeks to months.
3. The numbers of sites required to overcome the first disadvantage described above, and the time required for any experimental period (in 2 above), both result in field trials being large experiments. Consequently, the time before a result is obtained is lengthy and the cost in terms of technical personnel, cleaning reagents and apparatus considerable.

ASSESSMENT PARAMETERS

Any of the factors described in Section 1.7.1 may be assessed in a field trial. However, the factors selected for measurement vary with the information required and the apparatus, resources and knowledge available.

1.7.3 LABORATORY ASSESSMENT OF CLEANING PERFORMANCE

Laboratory techniques have the advantages of studying cleaning systems under precisely controlled conditions, with small samples for relatively small costs. These techniques may be divided into those in which the soil and cleaning solutions are manually applied to the test pieces, or those using devices ("simulators") to automatically apply the solutions. The fact that the amount of residual soil and number of micro-organisms are insignificant after a single soiling/washing cycle has made the application of the system for multiple cycles necessary in order to permit soil and organisms to accumulate to a level where they are easily assessed.

MANUAL TECHNIQUES

There are many manual techniques for assessing the performance of components of the cleaning system, particularly detergency and sanitizing. However, only three studies have been reported where complete cleaning systems have been assessed by manual techniques. Clegg & Cousins (1970) examined the detergency of a system on test panels over 18 cycles by physical and chemical techniques. Mitchell & Clifford (1964) examined the detergency and sanitizing performance of systems on test panels over 7 cycles by bacteriological techniques. Bacic et al (1968) also used bacteriological assessment methods, but used bucket milking machine units, to which they applied the test

systems over 20 milkings.

These techniques permitted the examination of experimental treatments under defined and controlled laboratory conditions. However, the need to apply all the soiling and cleaning solutions manually made them extremely labour-intensive. As a result, they have been largely replaced by simulator techniques.

SIMULATOR TECHNIQUES

Simulators are laboratory testing units which apply soil and the components of the cleaning system to test pieces in a way which imitates practical conditions (Whittlestone et al. 1963).

Classification of Simulator Techniques

There are three basic types of simulator techniques:

1. The "single-cycle pre-soiled" apparatus was the original type developed and is the simplest procedure. Test panels are usually soiled in the laboratory, placed in the simulator and subjected to the washing system and the soil removal is then estimated. This type of simulator has been used by many workers in the soak-tank cleaning of textiles (Harris 1954) and by others investigating recirculation cleaning (Bourne & Jennings 1961; Jennings et al. 1957; Peters & Calbert 1960; White & Rabe 1970). Soil estimation must be extremely sensitive as the amount remaining after a single cycle is very small. For this reason radioisotope labelling of the soil has been widely used.
2. The "single-cycle soiling and washing" simulators are also simple items of equipment. In this technique the test surfaces are clean at the commencement of the simulator period and the slides are soiled and washed in the simulator. In this way a more "natural"

soil/cleaning system interaction takes place. Again, as only a single cycle is used, the small amount of residual soil can make soil estimation difficult. This type of simulator technique has been widely used (Burton 1961; Hankinson & Carver 1968; Maxcy & Shahani 1961).

3. The "multiple-cycle soiling-and-washing" simulators are usually quite complex items of equipment. The repetitive nature of the procedure has prompted most investigators to automate the operations required. In this technique the test surfaces are clean at the commencement of the simulator period,and the slides are soiled and washed once each cycle in the simulator. As a number of cycles occur before the test pieces are examined, the soil residue from one cycle becomes the substrate for the soiling in the subsequent cycle and, unless the cleaning system is perfectly efficient, soil accumulation will occur. Corrosion of the substrate may also result which permits evaluation of this important parameter. This is more closely related to the practical situation, as multiple cycles are used in industry and soiling occurs in a "natural" manner. This type of simulator technique has been used by a number of workers (e.g. Calbert 1958; Dunsmore et al. 1977; Lewandowski 1958; Murphy 1973; Nisbet & Langdon 1975; Peters & Calbert 1960; Whittlestone et al. 1963; Wildbrett & Kiermeier 1960).

Types of Simulators

The mechanical arrangements of simulators also fall into a number of classes:

1. Soak tanks: These comprise a single tank that may be fitted

with spray nozzles and pumps to simulate practical application methods. These are used widely for textile detergent assessment (Harris 1954).

2. Rotating Dippers: These machines consist of a number of tanks, each containing a component of the soiling and/or cleaning system, placed around the circumference of a circle, and into which the test pieces are serially immersed. These are relatively simple machines, when compared to other multiple cycle simulators. They are usually used to determine the chemical effects of cleaning, with no attempt to simulate the effects of physical factors, such as solution turbulence. Their major deficiency is that the same solution is used repeatedly for soiling or washing. This type of equipment has been used for 700 cycle corrosion evaluation (Wildbrett & Kiermeier 1960) and 15 cycle milkstone deposition studies, this latter work being performed on a machine designed for automatic histological staining (Lewandowski 1958). A 24 cycle machine for assessing milking machine cleaning systems has also been described (Dunsmore et al. 1977a).
3. Recirculation units: These are the most popular type of simulator. A number of simple, manually-operated types have been developed (Bourne & Jennings 1961; Calbert 1958; Hankinson & Carver 1968; Jennings et al. 1957; McGregor et al. 1954; Peters & Calbert 1960). The more complex type is the automatic multiple-cycle machine (Dunsmore et al. 1977a; Murphy 1973; Whittlestone et al. 1963). These machines require programmers, automatically-controlled valves and apparatus to dispense solutions. They may be used to simulate both the physical and chemical operation of the cleaning system under evaluation. Usually each solution is used only once and it

is then discarded, as in the practical situation. This overcomes one of the major deficiencies of the rotating dipper type of machine. The size of the simulator can vary greatly; Nisbet and Langdon (1975) achieved considerable miniaturisation with a simulator using radioisotopically labelled soil, while Rohleder (1970) and Terplan et al. (1969) described actual milking installations which were used in the manner of simulators.

Two methods of estimating the removal of the artificial soil have been used with the single-cycle simulators: gravimetric (Hankinson & Carver 1968; McGregor et al. 1954) and, where more sensitive results were required, radioisotopically labelled soils (Jennings et al. 1957; Nisbet & Langdon 1975; Peters & Calbert 1960).Peters & Calbert (1960) examined three methods of labelling the milk of the artificial soil with ^{32}P and found a radioactively labelled bacteriological culture gave satisfactory results, whereas both *in vivo* and *in vitro* radioactively-labelled milks were unsatisfactory. Jennings et al. (1957) also examined the labelling of the milk soil with ^{32}P and found that, in all cases, the correlation between the weight of soil removed and the radioactivity removed was extremely high for the soil examined. Bourne and Jennings (1961) also used ^{14}C-labelled sucrose and ^{14}C-labelled tristearin in pre-soiled simulator studies.

In soiling-and-washing simulators where estimation of milk deposits is required, the gravimetric technique is the most popular (Calbert 1958; Dunsmore et al. 1977a, Lewandowski 1958; Murphy 1973; Whittlestone et al. 1963; Wildbrett & Kiermeier 1960). The use of the gravimetric technique is facilitated by the accumulation of the deposit on multi-cycle simulators, as the large deposits provide improved accuracy of measurement. Nisbet and Langdon (1975) used

^{131}I-labelled protein to achieve very sensitive assessment of developing milk residues. Two groups have chemically analysed the deposit for a major constituent as a method of estimation. Burton (1961) used the biuret reaction to determine the amount of protein, while White and Rabe (1970) used an analysis for calcium as an index of total soil. Maxcy and Shahani (1961) applied a bacteriological assessment, using E. coli-labelled milk as the soiling medium, while White and Rabe (1970) used a natural milk culture. Terplan et al. (1969) and Rohleder (1970) used recovery of bacteria from natural milk contamination to assess detergent disinfectants.

Minor modifications of the procedure used for gravimetric assessment of deposits have also enabled a number of workers to measure corrosion effects. Wildbrett & Kiermeier (1960) used a rotating dipper for corrosion tests only, while others (Murphy 1973; Whittlestone et al. 1963; Whittlestone & Twomey 1966) have combined deposition and corrosion evaluation.

Applications of Simulator Testing

The flexibility and experimental control provided by the simulator technique has enabled it to be applied to many aspects of dairy cleaning, for example:

1. Ultra-high temperature processing equipment (Burton 1961);
2. Comparison of detergent types (Lewandowski 1958);
3. Examination of the effects of hard water quality and application temperature on the cleaning performance of detergents (Peters & Calbert 1960);
4. Comparison of milking machine detergent formulations (Dunsmore et al. 1977c; Twomey 1965);

5. Determination of the optimum operating conditions for detergents (Calbert 1958; Peters & Calbert 1960; Twomey 1965); and solution flow through pipelines (Bourne & Jennings 1961; Hankinson & Carver 1968; Jennings et al. 1957).
6. Comparison of complete cleaning systems for cleaning and corrosion performance (Twomey 1965), and cleaning and sanitizing efficiency (Maxcy & Shahani 1961; Rohleder 1970; Terplan et al. 1969; White & Rabe 1970).
7. Examination of fundamental factors in soiling and soil removal (Bourne & Jennings 1961; Nisbet & Langdon 1975) and some inter-relationships between components of a cleaning system (Lewandowski 1958).
8. Examination of the bacterial colonisation of surfaces (Twomey 1975).

Only one attempt has been made to use a simulator technique to assess detergents as part of a quality control programme run by a governmental authority (Murphy 1973). Here the test detergents are assessed within a standard cleaning system for their ability to control milk deposit formation, while minimising corrosion of the test surfaces.

Two studies have been reported in which, instead of using test surfaces to evaluate cleaning systems, the simulator technique has been used to apply standard cleaning systems to materials which require evaluation for dairy use. Kiermeier and Schattenfroh (1959) used a rotating dipper simulator to evaluate plastics for dairy pipelines and Maxcy and Shahani (1961) evaluated the cleanability of welded stainless steel pipelines for cleaning-in-place applications.

Advantages of the Simulator Technique

The advantages of simulator testing are (Whittlestone et al. 1963):

1. The testing may be rapidly performed. Months of field work may be reduced to days or hours of simulator work;
2. Control over the experimental situation. Conditions such as water hardness and solution temperatures can all be designed by the experimenter, and the variation between equipment and operators in the industry situation is overcome. This, in turn, produces a more accurate test result which permits improved differentiation between detergents;
3. The cost of testing is greatly reduced because the requirements for labour, detergents, sanitizers and other experimental equipment are reduced.

Disadvantages of the Simulator Technique

The disadvantages of the simulator technique are:

1. Many of the variables factors in the practical situation (e.g. water quality, soil, equipment design, cleaning apparatus, labour) are either standardised or eliminated in the simulator. This can limit the validity of the results, or their application to a variety of situations in the industry.
2. As simulators become more complex their design and reliable operation becomes an increasingly difficult task.
3. Trials showing significant correlation between the results of assessment by both simulator and field trial must be conducted before any laboratory results are of value.

2

INVESTIGATIONS INTO THE PERFORMANCE OF CLEANING SYSTEMS

2.1 THE SIMULATOR TECHNIQUE

The literature review in Section 1 demonstrated that considerable work has been done on the cleaning of milking machines. There is a great deal of information available on the theory and practice of detergency and similar knowledge on sanitizing. However, there is very little information on the way in which detergents, sanitizers and other cleaning system components interact in their effects on the physical, chemical and microbiological performance of the cleaning system. Consequently the development of new cleaning systems today is empirical, there being no general principles which can be applied.

This study was undertaken to investigate the following topics:

1. The contribution of each component of the system to the performance of the total system - assessed in terms of the control of soil accumulation and bacterial development on equipment surfaces;
2. The nature of the changes in the performance of cleaning systems with time;
3. The inter-relationships between the physical, chemical and biological characteristics of cleaning systems;
4. The cyclic changes which take place in the bacterial population

present on equipment surfaces, commencing with deposition from milk and finishing with contamination of the milk; and

5. The principles underlying the design of cleaning systems.

This study has been conducted at a time when an energy crisis has recently occurred and also when intensive dairying is being extended to many under-developed countries. These factors place constraints on the sophistication of the techniques which can be applied. Two such constraints in dairy hygiene are the lack of heating for sanitizing and expensive pumps and associated apparatus. For these reasons the test situation selected for study here was one in which cold chemical solutions were used for sanitizing, and the solutions were applied with minimum turbulence, as in "soak" cleaning systems .

2.1.1 CONCEPT OF THE TECHNIQUE

An understanding of the contribution of each component of a cleaning system to the performance of the total system can only be obtained by examining the substrate surfaces within each soiling/washing cycle and determining changes over many cycles. This type of study can be performed in the laboratory by using cleaning simulators which apply soiling and washing solutions to test substrates over many cycles. In such work a typical experimental run would commence with the simulator loaded with sufficient pre-cleaned test pieces to permit removal and evaluation of the pieces at the required times during the experimental period. A number of cycles of the total in the experimental run are selected as "sampling cycles" and within these, samples are removed after application of each component of the soiling and cleaning system (e.g. after soil-

ing, after the post-milk rinse, and the detergent wash). The test pieces thus removed are then used for the gravimetric determination of accumulated soil, the enumeration of viable surface bacteria, or any other parameter of interest. Differences between values obtained before and after a particular cleaning system component can give a measure of the contribution of the component to the total system performance.

As this work examined the performance of milking machine cleaning systems the 12-hourly soiling and washing frequency, which occurs with the usual twice/day milking, was adopted. Routine assessment of the bacterial performance of a cleaning system can be facilitated by dosing the soiling solution with a standard number of selected bacteria (Dunsmore and French 1978). The numbers of these organisms recovered from the test pieces after soiling and washing is taken as an index of the bacteriological control achieved by the system.

2.1.2 DESIGN OF THE SIMULATOR

The simulator was designed to permit repeated soiling and washing of test pieces over multiple cycles under automatic control. Large numbers of test pieces were loaded into the test cells before a run. They were soiled in milk which had been dosed with selected organisms and stored in a refrigerator, until pre-warmed immediately before soiling. The cleaning solutions were then placed in and drained from the test cell in the correct sequence.

The simulator was equipped with four test cells which were soiled with the milk from a common reservoir, but could have different cleaning systems applied independently. This was achieved by soil-

ing and washing the four test cells sequentially. The bacteriological condition of all solutions and gases was controlled to ensure that the soiling solution was the sole source of bacteria. The temperature and humidity of the atmosphere of the test cells were accurately controlled.

An overall view of the simulator is presented in Figure 2.1-1 and a diagram of the machine is presented in Figure 2.1-2. The diagram of solution flow in one test cell of the simulator in Figure 2.1-3 shows how the sequence of pre-milk solution, milk, and post-milk cleaning solutions are prepared in reservoirs, dispensed into the test cell and then run to waste.

The four test cells (41 - 44,Figure 2.1-2) containing the test pieces were fed with milk and cleaning solutions from a glass manifold (34). The milk was dispensed into the manifold from a vessel (8) which was supplied with milk at $4^{o}C$ from a reservoir (1) in the refrigerator above (2). Cleaning solutions were dispensed into the manifold from vessels (29 - 32) which were supplied with the solutions from reservoirs above (18 - 23). Dispensing of solutions was achieved by vacuum-controlled pinch valves operating on rubber tubes (e.g. 27, 33). The operations of all valves and agitators were controlled by the programmer (described below).

A view of the test cells is presented in Figure 2.1-4 and a diagram in Figure 2.1-5. These show the test cells (56) loaded with 130 test pieces (52) held on their edges. The valve-controlled solution inlet (54) was at one end of the cell and two outlets at the other. The cells contained 2 l of fluid when filled to the weir overflow (53) which was inserted in the end wall at a point 10 mm above the top of the test slides,to permit the removal of surface

Figure 2.1-1: General view of cleaning simulator

Key: 2. Refrigerator containing milk reservoir,

19. Reservoir containing cleaning solution,

31. Dispenser vessel for cleaning solution,

38. Water bath containing test cells,

47. Humidity box.

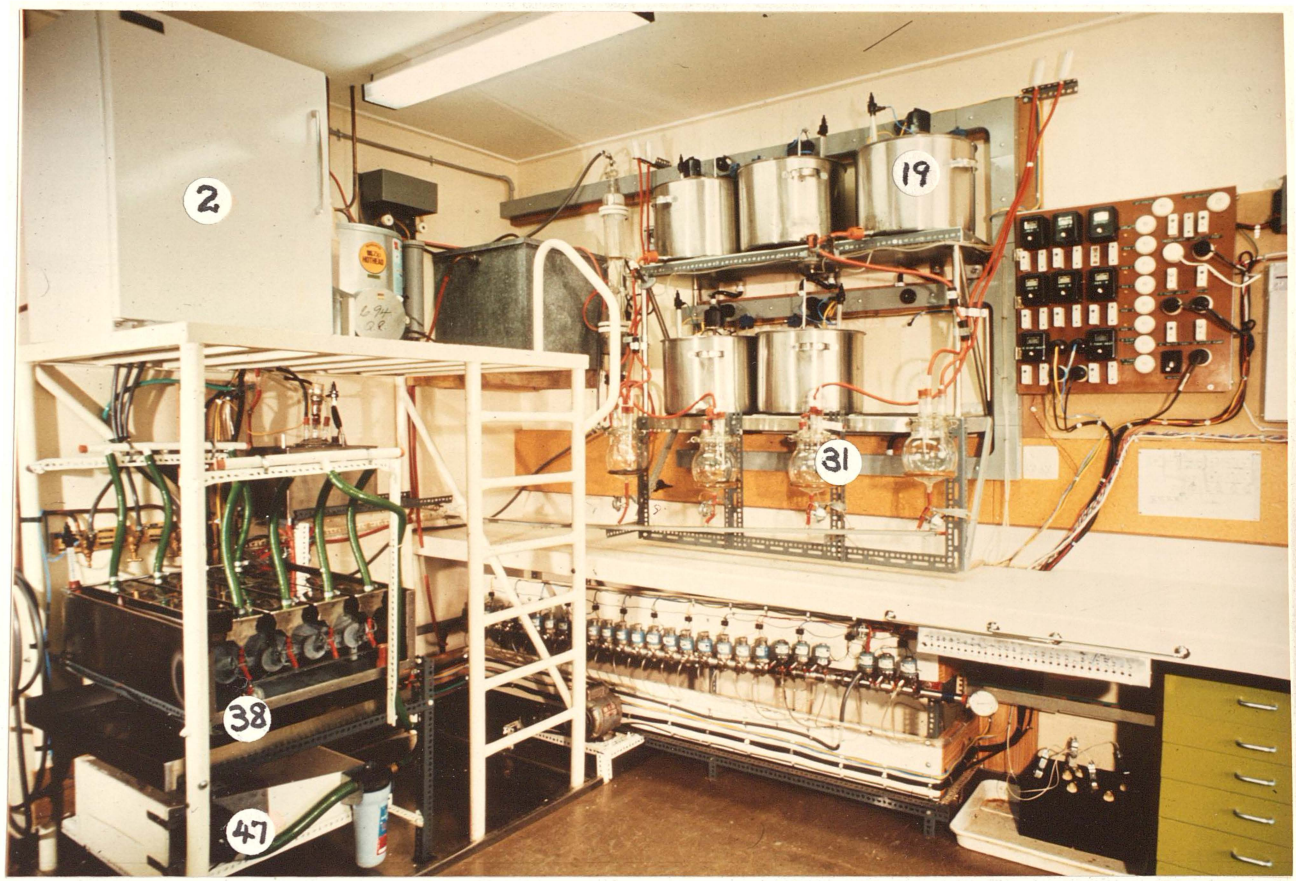

2
19
31
38
47

Figure 2.1-2: Diagram of cleaning simulator

Key:

1. Milk reservoir,
2. Refrigerator,
3. Milk inlet tube from reservoir to dispenser vessel,
4. Cleaning/steaming supply for milk inlet tube,
5. Breather tube for milk dispenser vessel,
6. Cleaning soln/steam inlet to breather tube,
7. Bacteriological filter on air inlet of breather,
8. Milk dispenser vessel,
9. Water bath for dispenser vessel,
10. Feed tube from milk dispenser vessel to inlet manifold,
11. Cleaning soln overflow on milk dispenser vessel,
12. Cold water supply to clean milk inlet system,
13. Detergent concentrate,
14. Hot water supply to clean milk inlet system,
15. Steam generator,
16. Tube feeding cleaning soln/ steam to milk inlet system
17. Steam tube to inlet manifold,

18 - 23. Cleaning soln reservoirs,

24. Cleaning soln agitator,
25. Contact thermometer,
26. Element heating cleaning soln,
27. Tube feeding cleaning soln to dispenser vessel,
28. Bacteriological filter on air inlet to dispenser breather tube,

29 - 32, Cleaning soln. dispenser vessels,

33. Valve controlling soln entry, to inlet manifold,
34. Inlet manifold,
35. Rubber sampling point,
36. Contact thermometer,
37. Heating element,
38. Water bath for test cells,
39. Agitator for water bath,
40. Drainage tray,

41 - 44. Test cells containing test pieces,

45. Outlet tube from weir overflow.
46. Outlet tube from test cell drain valves,
47. Box with soln to control atmospheric humidity,
48. Fan circulating humid air,
49. Bacteriological filter,
50. Tube supplying humid air to test cells.

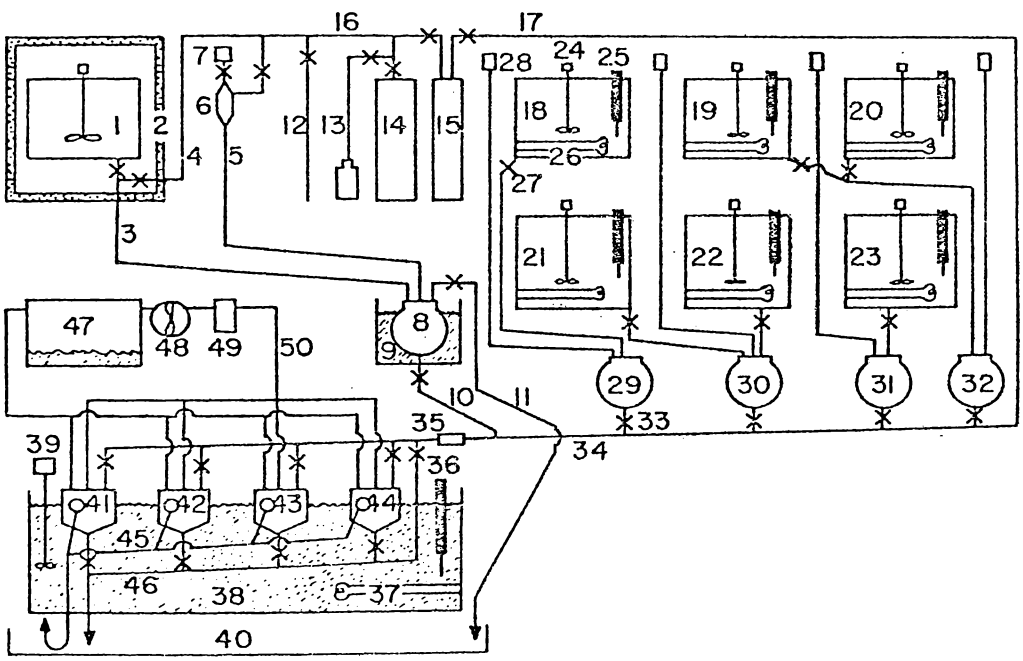
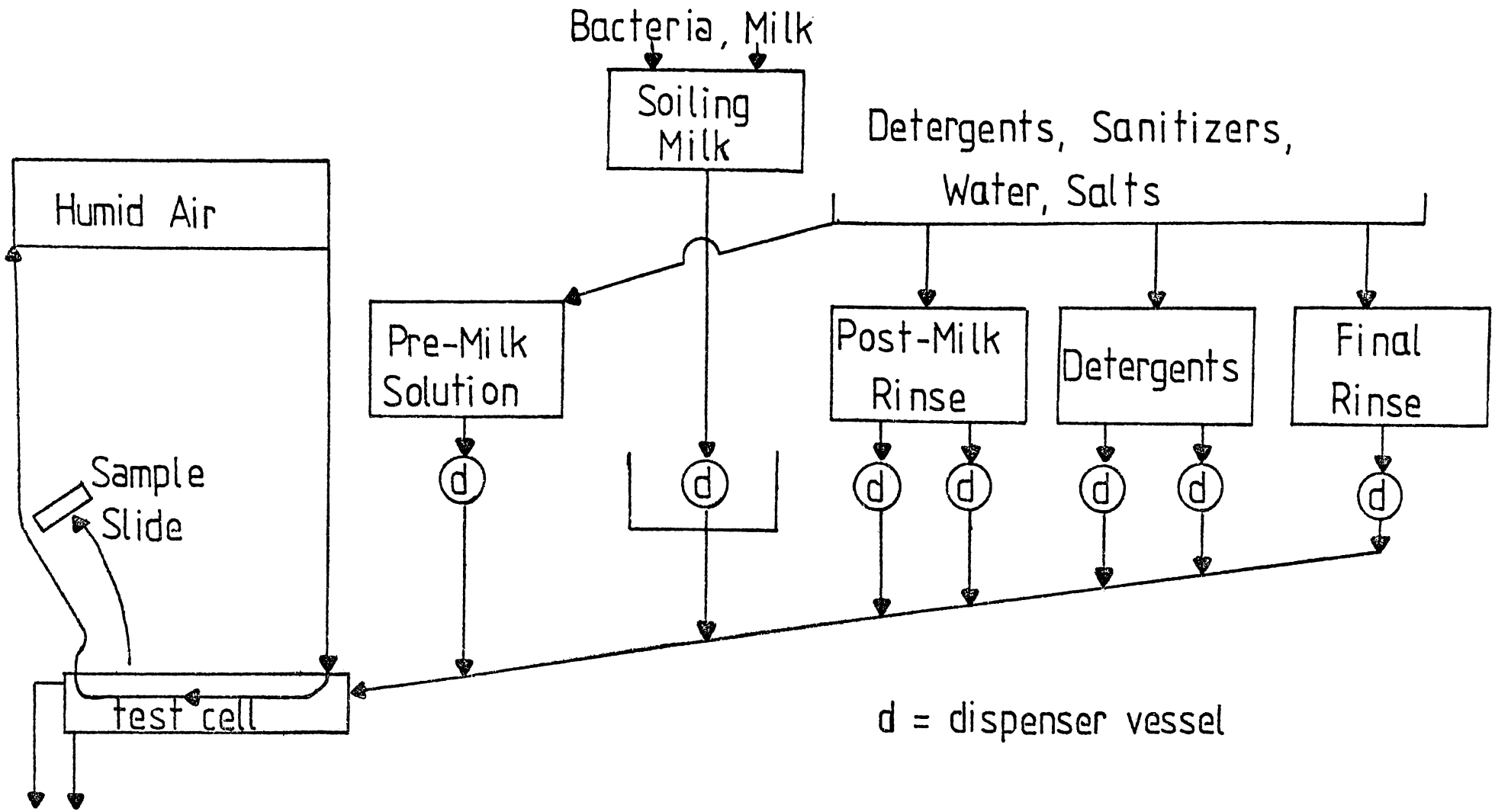

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Figure 2.1-3: Diagram of fluid flow in one test cell of the simulator


Bacteria, Milk
Soiling Milk
Detergents, Sanitizers, Water, Salts
Humid Air
Pre-Milk Solution
Post-Milk Rinse
Detergents
Final Rinse
Sample Slide
d
test cell
d = dispenser vessel
To waste

scum from the cleaning solutions. The valve-controlled outlet in the base of the test cell (55) was used to discard the solutions. Each test cell was sealed by four lids, each attached by spring clips,and was fitted with inlet and outlet tubes (51) through which air of constant temperature and humidity entered through bacteriological filters.

It is virtually impossible to achieve consistent treatment of large numbers of test pieces using a turbulent application of the cleaning solutions. Thus, the test cells were filled and emptied very gradually and no agitation was applied to the solution while in the test cell. This lack of turbulence had the advantages of removing one variable from the system and of reducing the efficiency of the cleaning system permitting a more rapid accumulation of soil, which reduced the time required for any experiment.

Figure 2.1-2 shows the test cells placed in a water bath (38) to maintain constant temperature. The humidity box (47) contained a tray with either water or a solution of a saturated salt to maintain constant humidity (ranging from 80 - 95% in different experiments). A fan (48) circulated the air first through a bacteriological filter (49) to the test cells and then returned it to the humidity box. The milk was held at 4°C in a 36 l stainless steel reservoir (1) in the refrigerator (2). The milk was supplied to the milk dispenser vessel (8), which controlled the volume of milk dispensed. The milk was brought to 30°C by a thermostatically controlled water bath (9) which surrounded the dispensing vessel.

The milk dispenser, milk inlet and dispenser breather tubes were soiled with milk every 3 h. To control contamination of these parts, they were automatically washed in two sections after

Figure 2.1-4: View of test cells of simulator

Key: 38. Water bath maintaining test cells at constant temperature

40. Drainage tray

51. Tubes supplying air of controlled humidity to test cells

52. Test pieces

56. Test cell.

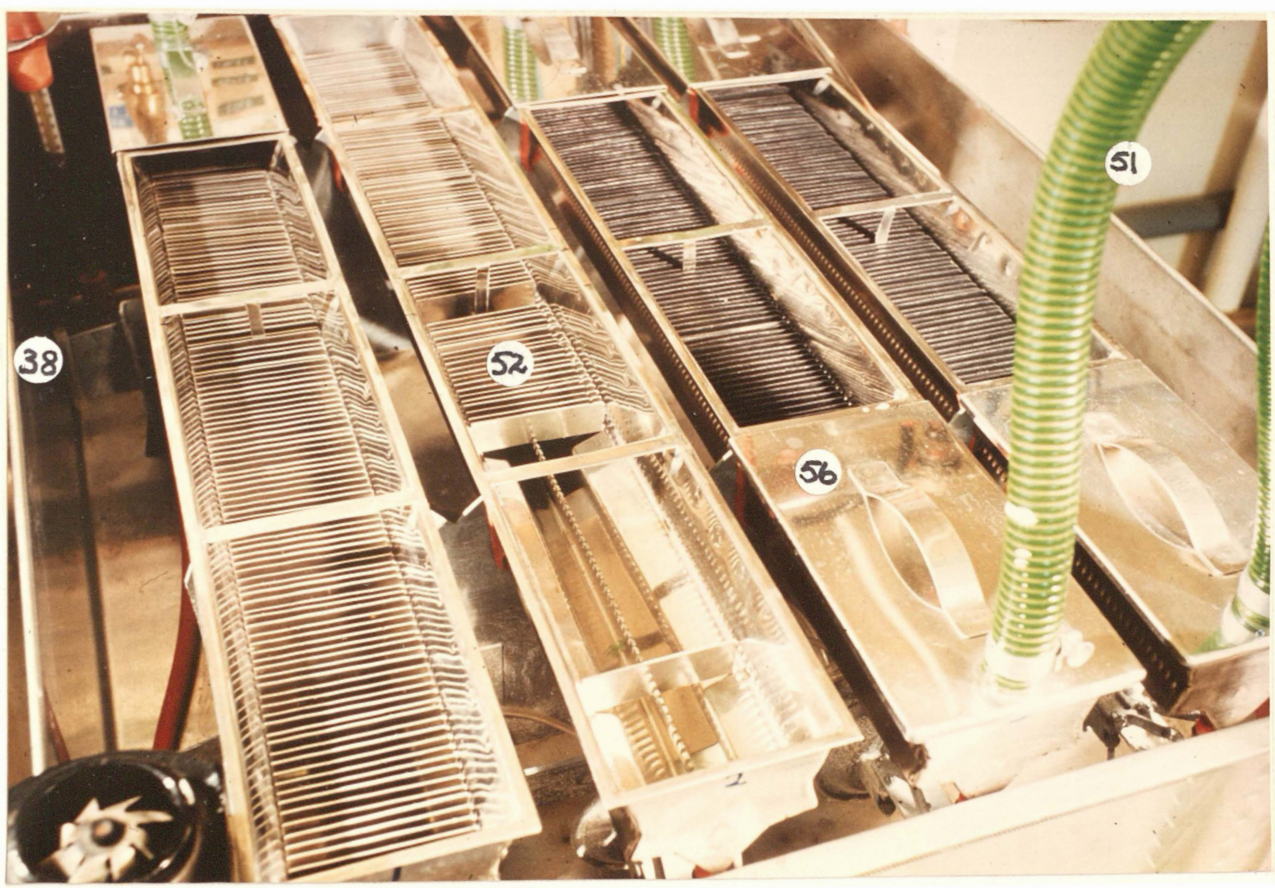

38
52
56
51

Figure 2.1-5: Diagram of test slides in test cell

Key: 38. Water bath maintaining test cells at constant temperature,

40. Drainage tray,

51. Inlet and outlet tubes for circulation of humid air through test cells,

52. Test pieces,

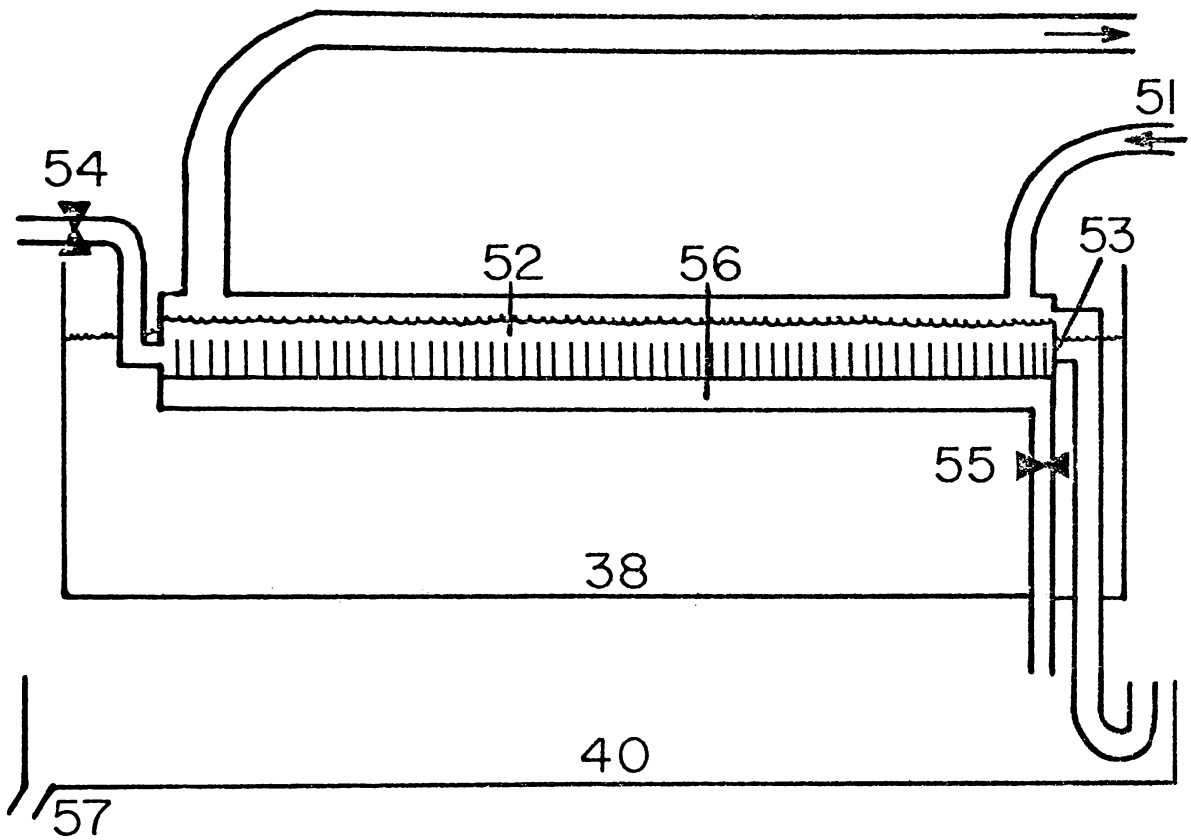
53. Weir overflow for cleaning solutions,

54. Inlet tube for soiling and cleaning solutions,

55. Valve controlling drainage from test cell,

56. Test cell,

57. Outlet from drainage tray.


51
54
53
52
56
55
38
40
57

each soiling by a cold water rinse and hot detergent, and were then steamed for 30 min at 100°C. The inlet manifold (34) was also steamed each cycle (17). The first section was washed by the cleaning solutions which entered the milk inlet tube (3) near the reservoir outlet valve (4), passed down to the milk dispenser and thence to waste. The second section was washed by the cleaning solutions which entered the glass vessel at the top of the milk dispenser breather tube (6) and passed down the breather tube into the dispenser vessel and thence to waste. The cold water was supplied from town supply through a solenoid valve (12). The hot detergent was prepared by mixing a concentrated detergent solution (13) with hot water (14) through a venturi. The steam was supplied at atmospheric pressure by an electric-powered generator (15).

Each of the 36 l stainless steel reservoirs containing the cleaning solutions (e.g. 18) was fitted with a reciprocating stainless steel paddle (24) driven by a programmable automotive 12 v motor. Constant temperature was achieved by an immersion element (26) controlled by a mercury contact-thermometer actuating a mercury relay (25). A Millipore* filter of 0.45 μm mean pore size was fitted to the tank breather (28). The cleaning solution was supplied to the dispenser vessel (e.g. 29) through the reservoir outlet valve (27). The solution volume dispensed was controlled by the depth of the breather outlet tube (28) in the dispenser vessel. The solution passed into the glass manifold (34) through the dispenser outlet valve (33).

The control of solution flow was achieved by pinch bars acting

* Registered trade name Millipore Corp.

on silicon rubber tubing. The bars were withdrawn to open the valve by a vacuum-operated diaphragm. The vacuum was supplied through tubing of 4 mm id. to the diaphragm valves by solenoid-actuated valves placed on a vacuum manifold maintained at 50 kPa (380 mm Hg). The timing of the actions of all solenoid valves and agitators in the cleaning solution reservoirs, milk reservoir and milk dispenser vessel, and the valves on the cleaning circuit for the milk dispenser system, was controlled by the program.

The programing of the simulator was achieved by the electronic command unit described by Jay (1978). This unit had a total cycle time of 3 h and actions could be operated at 21 s intervals. The unit performed four 3h periods before returning to the start point, giving the total cycle time for all test cells of 12h. The operations for each of the 3h periods were obtained from both a common program and a secondary program specific to each of the particular periods. Manual override switches could be used to control all programmed operations when necessary.

2.1.3 SIMULATOR OPERATION

The slides in each test cell were exposed to multiple cycles of 12h duration which included a 2.5h period of soiling and washing and a 9.5h intercycle or rest period. An example of the timing of the soiling and washing sequences is given in Table 2.1-1. For each test cell a 3h program-controlled period was provided for the soiling and washing operations. The remaining 9h of intercycle time were not controlled. The four test cells were sequentially soiled and washed during successive 3h periods so that all had been treated by the end of the 12h cycle time. The programmer then returned to the starting point and repeated the operations. Thus an experimental run

of 30 cycles would take 15 days to complete.

In preliminary work each test cell was filled and emptied through the same tube. The lack of turbulence permitted soil to be removed from the test pieces as the test cell was filling with solution, float on the surface of the solution and then redeposit on the test pieces as the solution was drained. As a result an unnaturally high soil level accumulated on the surfaces of the test pieces. This was overcome by dispensing two 2 l quantities of the post-milk rinses and detergent. The first quantity completely immersed the test pieces and filled the test cell to the level of the weir overflow. The second volume was then released from the dispenser, and filled the test cell to a level such that any scum was removed from the surface of the solution over the weir. The remaining solution was then drained away. This procedure overcame the problem of redeposition.

When preparing the simulator for a new experiment, all flexible hoses were replaced. All glass tubes and vessels and stainless steel reservoirs were autoclaved. The simulator was then reassembled and each section steamed at 100°C for 1h.

All cleaning solutions were prepared at the concentration at which they were to be applied to the test pieces using de-ionized water. Calcium chloride and magnesium sulphate were added to give a total hardness of 200 mg/l as $CaCO_3$, with a Ca:Mg ratio of 3:2. The milk used for soiling was whole milk obtained directly after pasteurising and chilling from the local dairy factory. The milk reservoir was filled, dosed with the required organisms and then placed in the refrigerator. All soiling and cleaning solutions were replenished at least once every 48h during a run.

Table 2.1-1: Sample program for soiling and washing each test cell

Approx. time from zero (min.)	Action
0.00 - 12.00	Supply milk to milk dispenser and heat.
0.00 - 12.00	Dispense pre-milk sanitizer - fill test cell - drain test cell.
12.00	Dispense heated milk into test cell in order to soil test pieces.
15.00 - 70.00	Cold-water rinse, hot-detergent wash and steam milk dispenser system (milk supply and milk breather tubes and milk dispenser vessel).
43.00	Drain milk from test cell - permit test pieces to air dry.
68.00 - 90.00	Dispense post-milk rinse, fill test cell - dispense second amount of rinse to overflow cell, then drain rinse from test cell.
96.00 - 120.00	Dispense detergent, fill test cell - dispense second amount of detergent to overflow cell - then drain detergent from test cell.
128.00 - 150.00	Dispense final rinse and fill test cell - then drain rinse from test cell.

2.1.4 PREPARATION OF TEST PIECES

Three types of substrate materials were used in the form of 75 x 25 x 1 mm test pieces ("slides").

1. Type 304 stainless steel with a 2B finish (Werra 1974);
2. Rubber of the same formulation as that used for teat cup liners, with additives for increased stiffness;
3. Glass microscope slides.

All slides were cleaned before use in the following manner: swabbed with soap and brush in cold water, rinsed in cold tap water, placed in a stainless steel washing box and boiled in 1% H_3PO_4 for 2 min., rinsed in 10 l of hot tap water, boiled in 1% NaOH for 2 min., rinsed twice in cold distilled water, boiled in distilled water for 2 min. and allowed to air dry.

The slides used for gravimetric assessment were weighed, and then all slides were placed in the test cells. The steel and glass slides were usually sterilised in the test cells in an oven at 160°C for 2h. When rubber slides were included all surfaces were sterilized by immersion in 70% (v/v) aqueous ethanol solution for 10 min. at room temperature.

2.1.5 GRAVIMETRIC ASSESSMENT OF ACCUMULATED SOIL

The mass of deposit accumulated with a treatment was determined by placing pre-weighed slides into the simulator, withdrawing them at the appropriate time in the experimental run, re-weighing them and determining the difference. All slides used for this gravimetric assessment were identified by a sequence of small marks on their edges.

All weighing was performed on a balance accurate to $\pm$ 0.01 mg

located in a sealed box with constant temperature and humidity. The temperature of 25°C was achieved by a thermostatically-controlled infra-red lamp. The relative humidity of 33% was achieved by placing 10 l of a saturated solution of $MgCl_2.6H_2O$ in the box (Young 1967). The solution was kept agitated.

After pre-cleaning, slides used for gravimetric assessment were placed in the balance box for 24h and then weighed. They were then placed in the test cells for experimental treatment. After withdrawal from the test cells they were stored in a desiccator containing silica gel until the experiment was completed. They were then placed in the balance box for 24h and re-weighed. All slides for any experiment were weighed at one session before an experiment, and at another session after the experiment to minimise any day-to-day variation.

2.1.6 DOSING OF SOILING MILK WITH BACTERIA

For the bacteriological results of different simulator runs to be comparable, the soiling milk must contain constant numbers of contaminating organisms. As any technique for sterilising milk in large volumes has undesirable effects upon the physico-chemical properties of the milk, pasteurised milk was selected as the soil. Pasteurised milk receives only a minimal heat treatment, and contains low numbers of thermoduric organisms (10^2 - 10^4/ml). By dosing with high numbers of the selected organisms ($\sim 10^6$/ml), it was hoped that the major effects would be due to these added organisms rather than the resident population of thermodurics.

On the basis of previously published data on the microflora of deposits on milking machines (e.g. Thomas & Thomas 1977) five organ-

isms were selected for study as possibilities for the two dosing organisms required. No spore-forming organisms were selected due to the difficulty of controlling them under the conditions of simulator operations. The organisms selected were: Streptococcus faecalis (ATCC 8043), Micrococcus luteus (NCTC 2665), Enterobacter aerogenes (NCIB 418), Pseudomonas aeruginosa (NCTC 674a), and Escherichia coli (isolated from milk). Each of these organisms was examined for three characteristics considered important in the simulator experiments; resistance to sanitizers, adherence to surfaces and survival under refrigeration.

SANITIZER RESISTANCE

The resistance of all organisms to three concentrations of each of three sanitizers was determined by the International Dairy Federation Suspension Test (International Dairy Federation 1962). The iodophor used (2% I, 16% H_3PO_4) was supplied by Economics Laboratory, NZ Ltd and applied at 6.3, 12.5, and 25.0 mg available iodine (Av. I)/l. An acidic quaternary ammonium compound (QAC - alkyl dimethyl benzyl ammonium chloride) supplied by Ivon Watkins-Dow Ltd, NZ was applied at 50, 100 and 200 mg QAC/l. The third sanitizer was chlorhexidine supplied by I.C.I.A.N.Z. Ltd and applied at 3.8, 7.5 and 15.0 mg chlorhexidine/l.

The $\log_{10}$ numbers of each organism surviving the various sanitizer treatments are presented in Figures 2.1-6a, 6b and 6c. Comparison of the results at 12.5 mg Av.I/l showed that Ps. aeruginosa was more resistant than E. coli and M. luteus, which in turn were more resistant than S. faecalis and E. aerogenes. The results at 100 mg QAC/l showed Ps. aeruginosa and S. faecalis to be

more resistant than E. aerogenes and E. coli, which were more resistant than the M. luteus. The data at 7.5 mg chlorhexidine/l showed the same ranking as the 100 mg QAC/l.

SURFACE ADHERENCE

The aim of this experiment was to compare the ability of the organisms to adhere to surfaces in the presence of a milk type of soil. This was determined by inserting clean glass slides into a milk solution containing the organism under study for up to 12h. The adherence was determined by removing the slide, rinsing it and counting numbers of adhering organisms.

Skim milk was selected as the soiling solution because of its thermal stability during sterilisation. It was inoculated with the organism and stored in 1500 ml beakers in the refrigerator overnight. Ten glass slides were pre-cleaned by the method described in Section 2.1.4, placed in a slide holder and sterilised by autoclaving at 112°C for 20 min.. A beaker of the milk was warmed at 30°C for 90 min. and then placed in an agitating water bath. The slide holder was placed in the milk and moderately agitated for 2h. As a reasonably constant number of contaminating organisms was required, and the numbers in the milk soil increased rapidly, the slides were changed to new beakers of milk freshly warmed to 30°C, after 2, 5, 8 and 11 hours of immersion.

Duplicate slides were removed from the soiling solution after 1, 5, 10, 20, 45, 90 min. and 3, 6, 9 and 12h. Each slide was then rinsed by sequential immersion in three test tubes each containing 75 ml sterile peptone water. One surface of the slide was then covered with 2 - 3 ml of tryptone yeast glucose agar (TYGA) containing

Figure 2.1-6a: Relative resistance of 5 organisms to three concentrations of an iodophor

Key: 1. *P. aeruginosa*
2. *M. luteus*
3. *E. coli*
4. *E. aerogenes*
5. *S. faecalis*

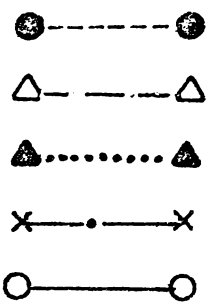
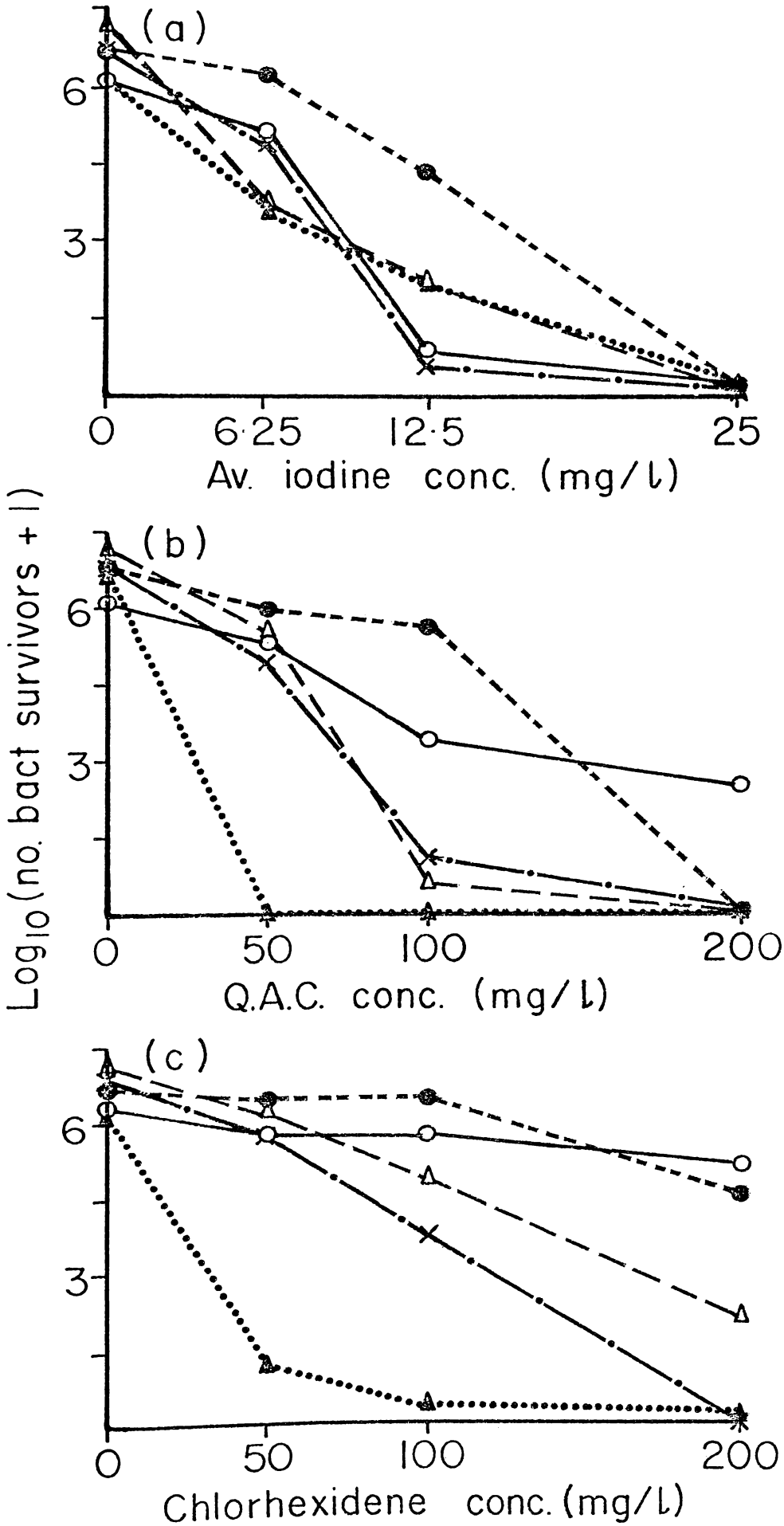


Figure 2.1-6b: Relative resistance of 5 organisms to three concentrations of a QAC.

Key: As for 2.1-6a

Figure 2.1-6c: Relative resistance of 5 organisms to three concentrations of a chlorhexidine.

Key: As for 2.1-6a.


(a)
6
3
0
6·25
12·5
25
Av. iodine conc. (mg/l)
(b)
6
3
0
50
100
200
Q.A.C. conc. (mg/l)
Log10 (no. bact survivors + 1)
(c)
6
3
0
50
100
200
Chlorhexidene conc. (mg/l)

1% triphenyl tetrazoliumchloride (TTC). The slide was then placed in a Petri dish with a swatch of wet cotton wool and incubated at 30°C for 24 - 48 hrs. The micro-colonies which developed were then counted using a low power binocular microscope. The soiling milk was also sampled, and plate counts performed using TYGA.

The numbers ($\log_{10}$ colony count + 1) on the glass surfaces are presented in Figure 2.1-7a and a typical pattern for the counts of the milk is shown for one organism (E. coli) in Figure 2.1-7b. There are real differences in the adherence characteristics of the organisms (Figure 2.1-7a) which are not a reflection of the changes in numbers in the soiling milk (Figure 2.1-7b). Ps. aeruginosa showed a three-stage attachment curve, adhering in low numbers initially, having a moderate adherence rate in the period from 2 to 9h and an accelerating rate of adherence at 12h. It reached a maximum density of 22,000 colonies/cm^2 at 12 h, the greatest of all organisms. E. coli adhered in high numbers initially, but this number increased very little during the rest of the 12 hour period. The coccoid organisms S. faecalis and M. luteus both had similar patterns, increasing from very low initial numbers to a constant level after 2 - 3h. No E. aerogenes organisms adhered in the first 60 min., a few during the period up to 6h, but the rate of adherence was greatest in the 6 - 12h period.

REFRIGERATOR STORAGE

The milk used for soiling was dosed with the contaminating organisms and placed in a refrigerator for a 48h period, during

Figure 2.1-7a: Relative adherence of 5 organisms to glass in a skim milk soil

Key:
1. *P. aeruginosa*
2. *M. luteus*
3. *E. coli*
4. *E. aerogenes*
5. *S. faecalis*

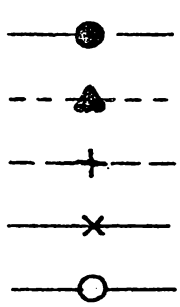
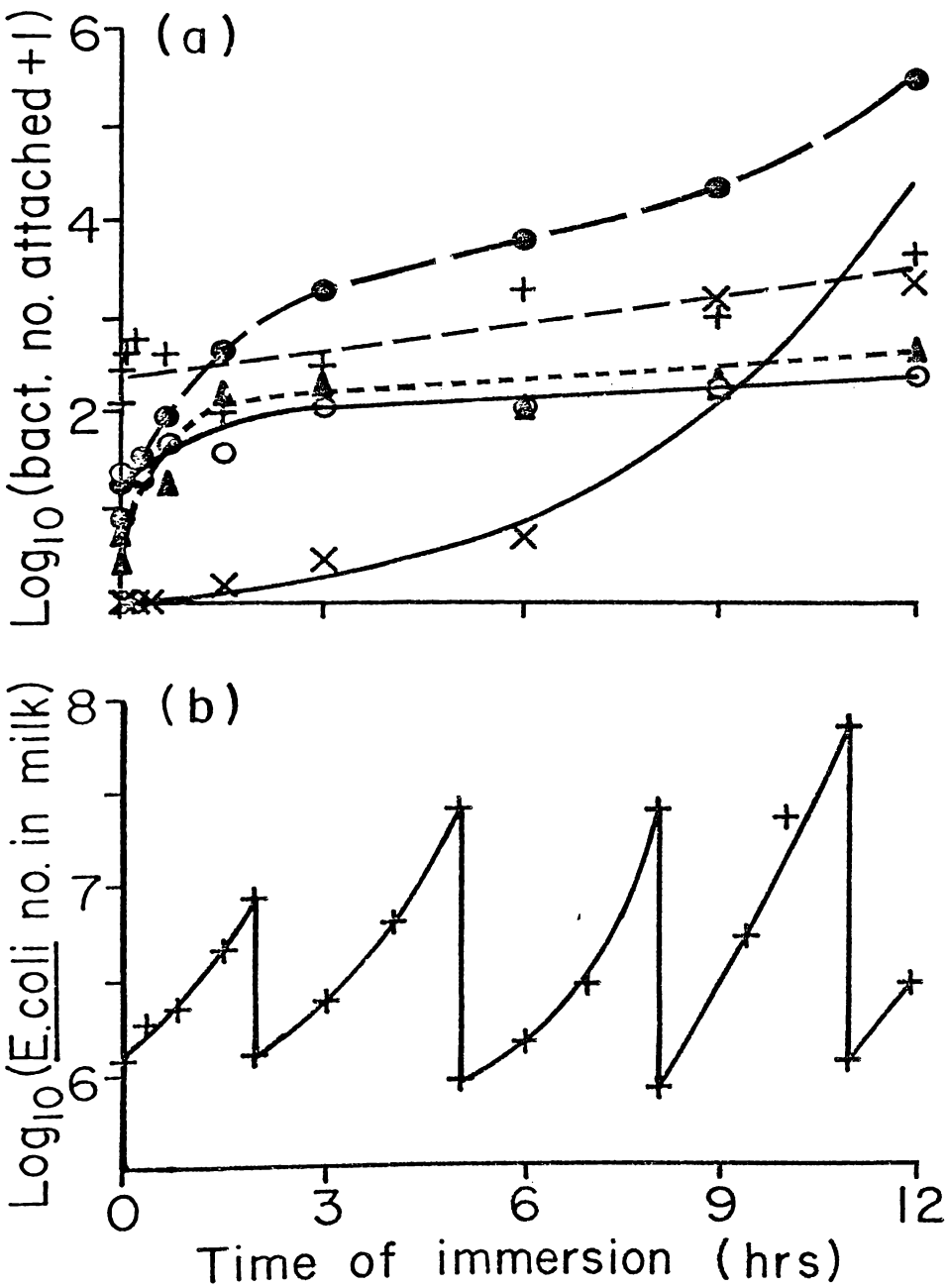


Figure 2.1-7b: Change in numbers of *E. coli* in soiling solution of skim milk over time


(a)
Log10 (bact. no. attached +1)
6
4
2
(b)
Log10 (E.coli no. in milk)
8
7
6
0
3
6
9
12
Time of immersion (hrs)

which time it was gradually dispensed for soiling. It was important that the growth characteristics of the dose organisms were similar throughout the 48h that one quantity of milk was used. This was assessed by inoculating pasteurised milk with either one or two cultures and then storing it in a refrigerator for 48h. Samples were taken at 0 and 48h refrigeration and incubated at 30°C for 5h. The bacteria were enumerated by plate counting on selective and TYGA media at 0 and 48h of refrigeration and after the 5h growth periods at the beginning and end of refrigeration. The organisms used were S. faecalis, Ps. aeruginosa, E. aerogenes and E. coli singly, and S. faecalis paired with either Ps. aeruginosa, E. aerogenes or E. coli. The selective media used were modified Edwards* for S. faecalis and Violet Red Bile Agar* (VRBA) for E. aerogenes and E. coli (American Public Health Association 1965).

The counts obtained on the selective media are presented in Table 2.1-2. These show that the only organism to decrease in numbers significantly during the refrigeration period was E. aerogenes, and this did not occur when paired with S. faecalis. All organisms showed slightly slower growth after being refrigerated, but the only significant difference was with E. aerogenes when paired with S. faecalis.

SELECTION OF ORGANISMS FOR DOSING

For ease and relaibility of experimentation it was decided to dose the milk with two types of organisms. It was desirable for these organisms to represent as wide a range of types as possible, and also to be fairly resistant to the cleaning system being applied. Informat-

* Registered trade name - Oxoid

Table 2.1-2: The effect of refrigeration for 2 days on the growth and survival of single and paired organisms

Culture	Org.	Log_{10} colony count				Change in log_{10} colony count		
		Before initial growth and refrigeration	After initial growth	After refrigeration and before final growth	After final growth	Initial growth	Refrigeration	Final growth
S. faecalis	S	5.63	5.42	5.76	5.38	-0.21	0.13	-0.38
Ps. aerug.	P	6.22	7.98	6.15	6.88	1.76	-0.07	0.73
E. aerog.	Ea	4.40	6.31	3.24	4.43	1.91	-1.16	1.19
E. coli	E	4.09	6.97	4.52	6.90	2.88	0.43	2.38
S. faecalis + *Ps. aerug.*	S	5.60	5.88	5.67	6.88	0.28	0.07	0.41
	P	6.40	7.38	6.37	6.84	0.98	-0.03	0.47
S. faecalis + *E. aerog.*	S	5.61	5.40	5.57	6.00	-0.21	-0.04	0.43
	Ea	4.41	6.29	4.52	4.85	1.88	0.11	0.33
S. faecalis + *E. coli*	S	5.42	5.47	5.73	5.04	0.05	0.31	-0.69
	E	4.43	6.99	4.35	6.08	2.56	-0.08	1.73

ion on the five selected organisms obtained from the preceding experiments and from the literature (Buchanan & Gibbons 1974) is presented in Table 2.1-3. The relative resistance to sanitizer of the organisms was arbitrarily classified into categories ranging from "not resistant", to "very highly resistant". The ability of the organism to survive refrigeration and grow satisfactorily afterward was classified as either "satisfactory" or "good". The surface adherence of the organisms is described according to the initial adherence and the changes over the 12h period.

The Gram-positive organisms were similar in most characteristics, but S. faecalis was selected because of its significantly greater resistance to all of the sanitizers applied. Thus a strict aerobe with poor surface adherence had been selected. To complement this either E. coli or E. aerogenes could be used as they are both facultative anaerobes with reasonable, but different,surface adherence. Ps. aeruginosa was omitted because it is an aerobe, and could give problems both in producing constant doses and in measurement of optical density due to pellicle formation during growth. In the simulator experiments S. faecalis, E. coli and E. aerogenes were used as dosing organisms in different combinations.

DOSING TECHNIQUE

As a total count of 10^6 bacteria/ml was required in the dosing milk, approximately 5×10^5 of each of the two dose organisms was required. This was achieved by the following technique for each organism:

1. A slope of TYGA was inoculated from a stock culture and incubated at $30^{\circ}C$ for 20h.

Table 2.1-3: Comparison of 5 organisms for those characteristics relevent to dosing

Data	Escherichia coli	Enterobacter aerogenes	Pseudomonas aeruginosa	Streptococcus faecalis	Micrococcus luteus
Cellular morphology	rod, singly or paired	rod, single	rod, single, paired or small chains	coccus, pairs or chains	coccus, single, paired, regular or irregular clusters
Motility	peritrichous flagella	non-motile (usually)	polar flagellum	occasionally motile	non motile
Gram reaction	negative	negative	negative	positive	positive
Temp. minimum (°C)	4	12	4	10	10
optimum (°C)	37	35	37	37	30
maximum (°C)	45	43	42	45	45
Catalase formation	occasionally	yes	no	no	no
Respiration	facultative anaerobe	facultative anaerobe	aerobe	facultative anaerobe	strict aerobe
Growth in broth	uniform turbidity	uniform turbidity	turbidity with thick pellicle	no pellicle formed, turbidity then settling	uniform turbidity then clearing
Sanitizer resistance:					
- Iodophor	moderately resistant	slightly resistant	resistant	slightly resistant	moderately resistant
- QAC	slightly resistant	slightly resistant	highly resistant	resistant	not resistant
- chlorhexidine	highly resistant	resistant	very highly resistant	very highly resistant	slightly resistant
Refrigeration survival and growth	good	satisfactory	good	good	no information
Surface adherence	immediate adherence in high numbers	no initial adherence, rapid after 6 hrs	low initial numbers, but rapid increase to high numbers	initial increase to low static numbers	initial increase to low static numbers

2. The slope was washed with 5 ml of a sterile 0.1% aqueous solution of peptone into a 500 ml conical flask fitted with a glass side-arm, containing 100 ml TYG broth.
3. The broth culture was placed in a shaking incubator at 30°C until the optical density reached 0.3 ± 0.03. This was equivalent to 5×10^8 organisms/ml.
4. Thirty six ml of the broth culture was placed in the 36 l of soiling milk to give a dose of 5×10^5/ml of each organism.

2.1.7 ENUMERATION OF BACTERIA

SURFACE COUNTS OF TEST PIECES

An estimate of the numbers of viable organisms present on the slides removed from the simulator was achieved by a swabbing/colony count technique. All surfaces of the slides were swabbed with two swabs which had been moistened in 10 ml of solution designed to inactivate any residual sanitizer (International Dairy Federation 1962). The solution contained sodium thiosulphate for halogens, Tween 80 and lecithin for other sanitizers and 0.1% peptone. The swabs were broken off into the inactivator solution and agitated 10 times manually.

Colony counts were performed on a selective medium for each dose organism, and also on TYGA. Duplicate plates were used for each dilution. The plates were incubated for 48h at 30°C before counting. The selective media used were VRBA for *E. coli* or *E. aerogenes* and TYGA with sodium azide (0.4 g/l) for *S. faecalis*. The $\log_{10} (x + 1)$ transformation was applied to all mean colony counts before analysis.

The efficiency of this swab recovery technique was determined by swabbing a number of experimental slides and then immersing them

in TYGA and counting the micro-colonies after 24h. The number in the recovery solution is compared to the total number for recovery solution and slide in Figure 2.1-8. It can be seen that the recovery efficiency increased as the numbers on the original slide increased. Recovery was approximately 85% at 100 organisms/slide and 99% at 10,000,000 organisms/slide. Thus the swab/colony count as performed provided an accurate assessment of the numbers of viable organisms on the substrates.

CONTAMINATION COUNT OF TEST PIECES

Immediately before a surface is soiled there may be some live organisms on the surface. Some of these organisms may become incorporated into the soiling solution and become contaminants of the milk. It is the task of the cleaning system to minimise the number of these organisms.

To assess the number of such organisms, some slides were removed from the test cell immediately prior to soiling. They were then placed in 70 ml 0.1% peptone water in a test tube and inverted 6 times. The numbers of organisms in the recovery solution were determined as for the surface counts.

2.1.8 CLASSIFICATION OF SURFACE MICROFLORA

To determine the dominant organisms in any sample,up to 30 representative colonies from a plate were taken and grown on TYGA at 30°C. After 24h Gram stains were performed (Preston & Morrell 1962) and the organisms classified according to the scheme displayed in Figure 2.1-9 (Buchanan and Gibbons 1974). Each organism was tested for catalase (Clarke & Cowan 1952), and oxidase (Kovacs 1956).

Figure 2.1-8: Efficiency of recovering organisms from test surfaces by double-swabbing technique

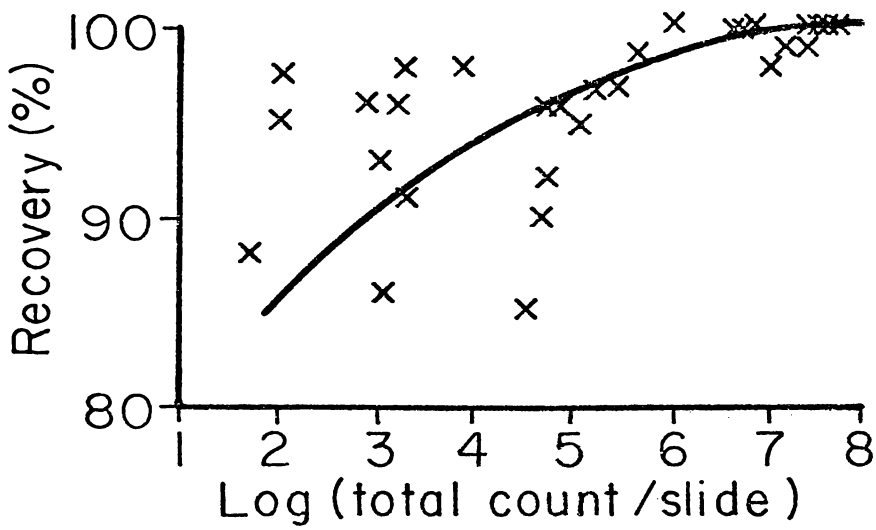
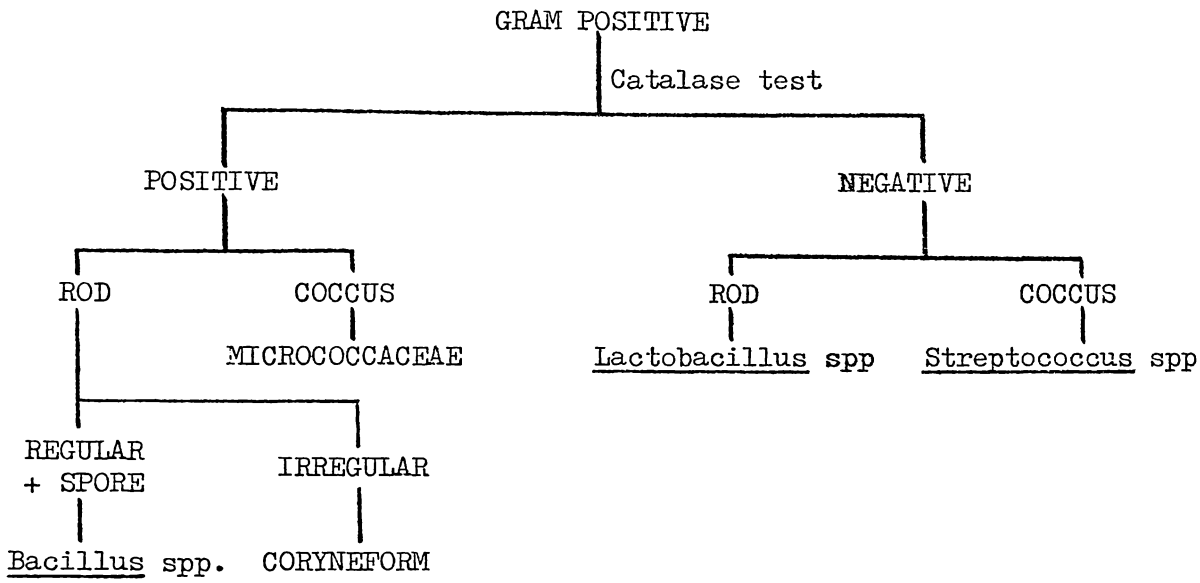
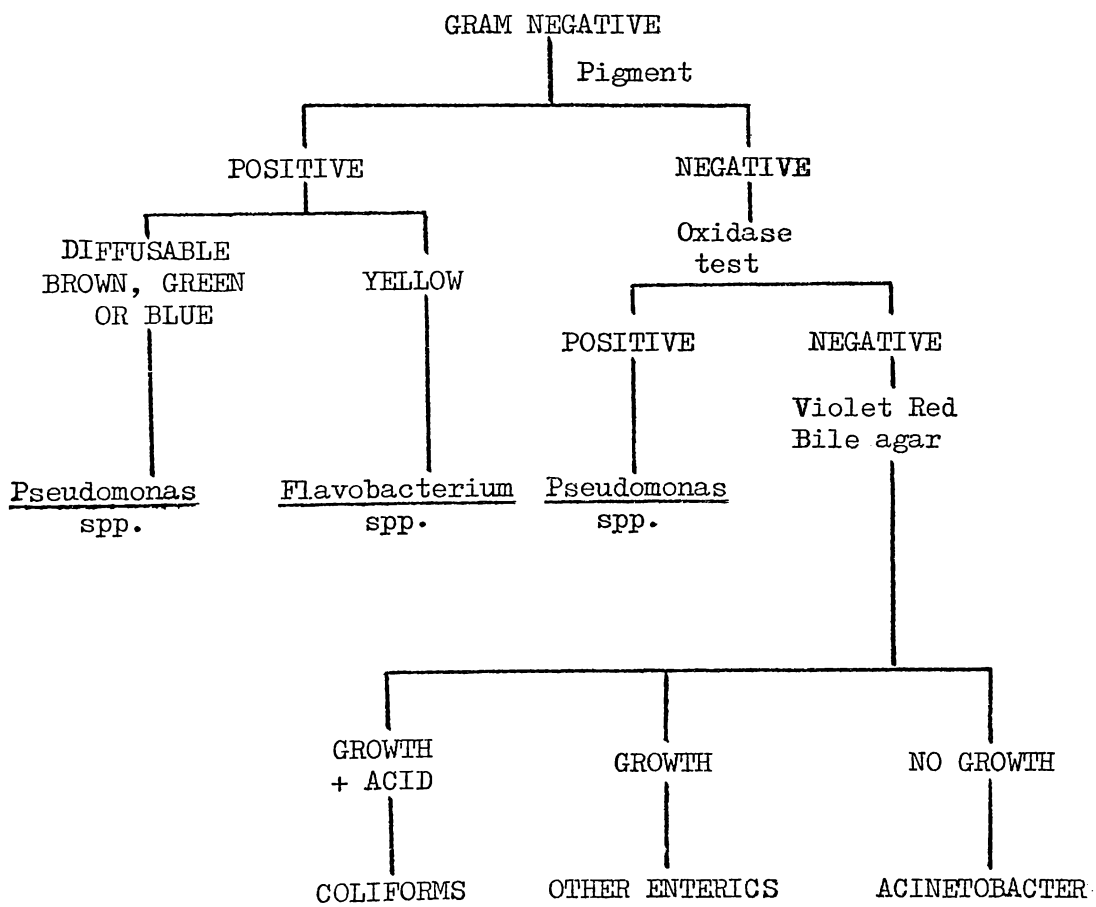

100
90
80
Recovery (%)
1
2
3
4
5
6
7
8
Log (total count /slide)

Figure 2.1-9: Classification scheme for surface microflora

(a) GRAM POSITIVE



(b) GRAM NEGATIVE



2.1.9 MICROSCOPY OF THE SURFACES OF SUBSTRATES

The soil and bacteria on substrate surfaces were examined by scanning electron microscopy (SEM). Small sections of the normal glass and rubber slides were used for this examination. Where stainless steel samples were required 25 x 4 mm strips were used made of type 304 foil with a 2B finish.

After removal from the test cell during an experiment the samples taken for examination by SEM were placed in 3% (v/v) aqueous glutaraldehyde for at least 12h. The following treatment was then applied - the slides were:

1. rinsed in three solutions of phosphate buffer, for at least 20 min. each solution;
2. fixed in 1% osmium tetroxide for 60 min;
3. rinsed in three solutions of phosphate buffer - as in 1 ;
4. dehydrated in a series of ethanol/water mixtures of the following compositions; 25% ethanol:75% water, 50:50, 70:30, 95:5, 100:0, 100:0 - the samples were immersed in each solution for at least 15 min;
5. Freon TF was then substituted for the alcohol by immersing the samples in a Freon TF/ethanol series of the following compositions 25% Freon TF:75% ethanol (v/v), 50:50, 75:25, 100:0, 100:0, - the samples were immersed in each solution for at least 15 min.;
6. stored in 100% Freon TF at 4°C until critical point drying;

The samples were then critical-point dried on Polaron E3000 apparatus and mounted on stubs with double-sided adhesive tape. They were then diode sputter-coated on Polaron E5000 equipment and then examined on a JEOL JSM-35 instrument, using an accelerating voltage of 35,000 Kv, gain bias 4 and a tilt of 30°.

2.2 SIMULATOR INVESTIGATION OF DEPLETION EFFECTS

The literature review revealed the lack of information on the contribution of individual components of the cleaning system to the performance of the total system. Also, the interrelationships between the removal and the killing of bacteria as methods of bacteria control have not been examined.

The experiment described here was performed to investigate:

1. The contribution of each system component to the performance of the total system;
2. The interrelationships between removal and killing of bacteria as control techniques;
3. The changes in performance of the cleaning system with time; and
4. The value of the simulator technique as a means of analysing cleaning system performance.

2.2.1 EXPERIMENTAL DESIGN

The milking machine cleaning system currently recommended in New Zealand and Australia is known as the "triple system" (Calder & Twomey, 1966; Whittlestone & Twomey, 1966). This is a system which uses heat sanitizing and an alkaline washing solution. The sanitizing effect of the hot alkaline detergent and post-wash rinse of boiling water are complemented by a pre-milk sanitizing with iodophor.

In this experiment, variations of the major components of the triple system were applied at lower temperatures. The components of each system are presented in Table 2.2-1. This shows that in the "triple" system (A.1) the milk soil was followed by a post-milk rinse of cold water. This rinse was followed by a warm alkaline detergent, which in turn was followed by the post-wash rinse of cold

Table 2.2-1: Components of the 4 experimental systems and sampling times in depletion study

Systems	System components					
	Pre-milk iodophor rinse (25 mg Av.I/l), 30°C	Milk soil	Post-milk rinse	Wash	Post-wash rinse	Intercycle time
A.1 Triple	+	+	Water 20°C	Alk. detgt, 3 g/l, 50°C	Water 20°C	-
A.2 No final rinse	+	+	Water 20°C	Alk. detgt, 3 g/l, 50°C	-	-
A.3 No post-milk rinse	+	+	-	Alk. detgt, 3 g/l, 50°C	Water 20°C	-
A.4 No detergent	+	+	Water 20°C	Water 50°C	-	-
Sampling times						
- Surface bact. count	*I	S	M	R	D	F
- Gravimetric*		G				

* I - bacterial count at end of intercycle period (prior to pre-milk rinse),
S - bacterial count after pre-milk rinse (prior to milk soil),
M - bacterial count after milk soil (prior to post-milk rinse),
R - bacterial count after post-milk rinse (prior to detergent),
D - bacterial count after detergent (prior to post-wash rinse),
F - bacterial count after post-wash rinse (commencement of intercycle period),
G - deposit weight after pre-milk rinse (immediately before milk soiling).

water. The intercycle (rest) period of approximately 9.5h commenced after the post-wash rinse and was followed by the pre-milk rinse of iodophor sanitizer. After the pre-milk rinse, the milk soiling completed one 12h soiling/washing cycle. System A.2 (no final rinse) was the same as system A.1, except that the final water rinse was omitted. System A.3 (no post-milk rinse) was the same as System A.1, except that the post-milk rinse was omitted. System A.4 (no detergent) was the same as System A.2, except that the detergent concentrate was omitted from the warm washing solution. The powdered alkaline detergent was "Alka-Klenz", supplied by Syndet (NZ) Ltd and applied as a 3 g/l aqueous solution. The iodophor "Klenz iodophor" (2% I_2, 16% H_3PO_4) was supplied by Economics Laboratory, NZ Ltd and applied at 1.5 ml/l ($\equiv$ 25 mg Av. I/l).

The experimental systems were applied to the stainless steel slides for 36 cycles of 12h each with sampling performed at cycles 1/2, 7/8, 17/18, 27/28 and 35/36. At each sampling cycle triplicate samples were taken for the surface bacterial counts and gravimetric assessment of soil at the points shown in Table 2.2-1.

The test cells were maintained at a temperature of 30°C and relative humidity of 80%. Streptococcus faecalis was used as the dose organism at a rate of 10^6 organisms/ml, incorporated into the soiling milk.

2.2.2 RESULTS

The changes in the masses of the deposits which accumulated on the slide surfaces over 36 cycles are presented in Figure 2.2-1. All systems had a period of rapid increase of deposit over the first two cycles, and then a linear increase at a lower rate. System A.4

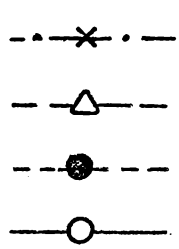
Figure 2.2-1: Accumulation of milk soil on stainless steel surfaces over 36 cycles with four cleaning systems in the depletion study

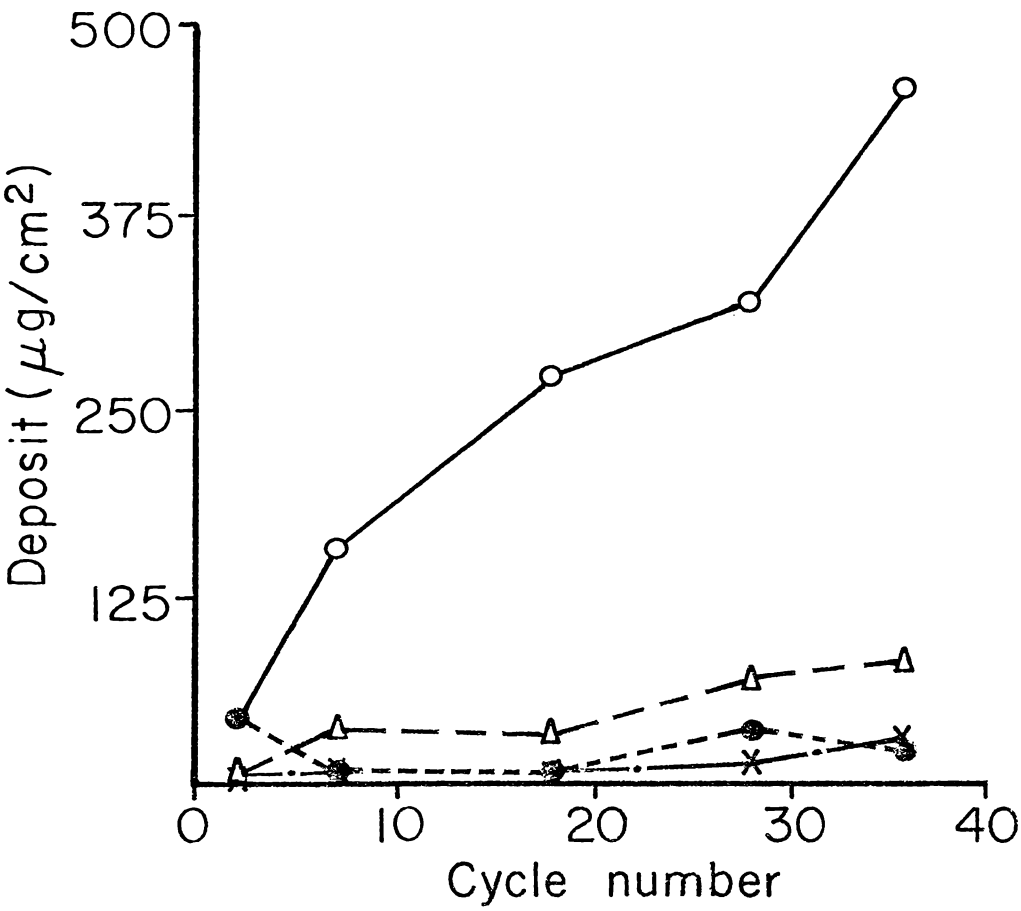
Key: Treatment A.1

Treatment A.2

Treatment A.3

Treatment A.4




500
375
250
125
Deposit (μg/cm2)
0
10
20
30
40
Cycle number

(no detergent) permitted the greatest accumulation of deposit, approximately 6 times that of the next greatest treatment (A.2 - no final rinse). This in turn resulted in twice as much deposit as system A.3 (no post-milk rinse). System A.1 (triple) was the most effective in terms of deposit, having slightly less than A.3.

The changes over 36 cycles in the numbers of viable S. faecalis organisms on the surfaces at the various stages of the cleaning system are presented in Figure 2.2-2a. The same information is presented three-dimensionally in Figure 2.2-2b. In this latter illustration, the count at the start of the post-milk rinse is the same as that after the milk soiling. Similarly, the count after the pre-milk sanitizer is the number on the surfaces immediately before a soiling. Thus, the changes in count traversing from left to right at any one cycle number show the reduction in count achieved by the components of the cleaning system at that cycle number. The same type of information is presented for Systems A.2, A.3 and A.4 in Figures 2.2-3, 2.2-4 and 2.2-5 respectively.

The effect of any particular component of the system or of the complete system in terms of the microbial populations on surfaces can be determined from two items of information; the numbers of viable organisms present on the surface after exposure to the component (survivors), and the change in the numbers caused by the component. Information in this form is presented in Figures 2.2-6 to 2.2-9. Figure 2.2-6a shows the number of S. faecalis remaining after the milk soil, while Figure 2.2-6b shows the numbers of organisms which adhered during the soiling operation at any time. Figures 2.2-6c, 2.2-7a, 2.2-7c, 2.2-8a and 2.2-8c show the numbers of survivors after exposure to the post-milk rinse, the detergent, the

Figure 2.2-2a: Changes in numbers of viable S. faecalis organisms on stainless steel surfaces after components of treatment A.1 (triple)

Key:
1. After milk soiling
2. After post-milk rinse
3. After detergent
4. After final solution
5. After intercycle period
6. After pre-milk sanitizer

Figure 2.2-2b: Changes in numbers of viable S. faecalis organisms on stainless steel surfaces after components of treatment A.1 (triple) - presented three-dimensionally

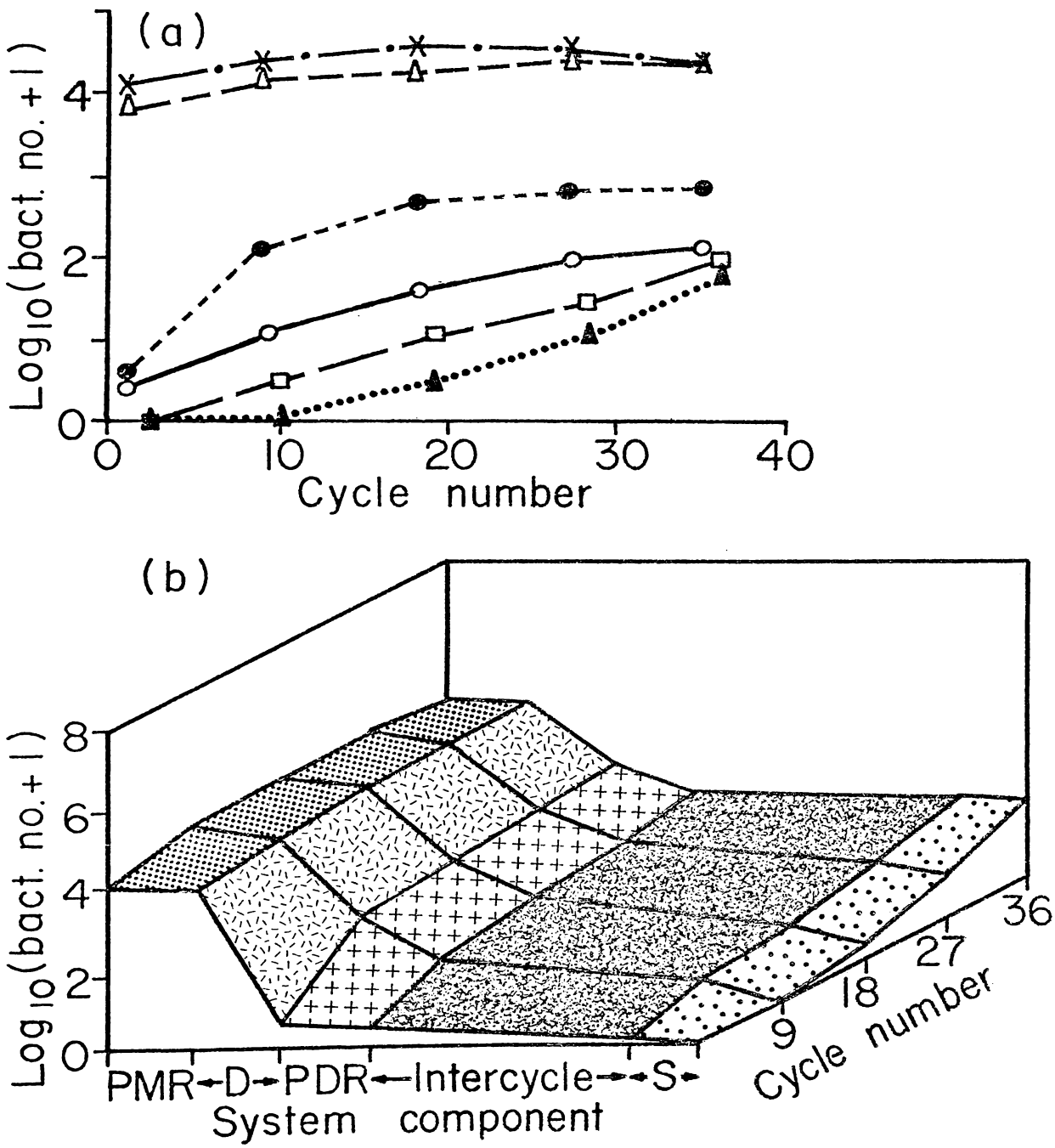

(a)
Log10(bact. no.+1)
0
2
4
0
10
20
30
40
Cycle number
(b)
Log10(bact. no.+1)
0
2
4
6
8
PMR
D
PDR
Intercycle
S
System component
9
18
27
36
Cycle number

Figure 2.2-3a: Changes in numbers of viable S. faecalis organisms on stainless steel surfaces after components of treatment A.2 (no final rinse)

Key:
1. After milk soiling
2. After post-milk rinse
3. After detergent
4. After intercycle period
5. After pre-milk sanitizer

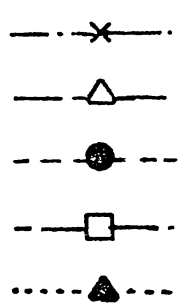
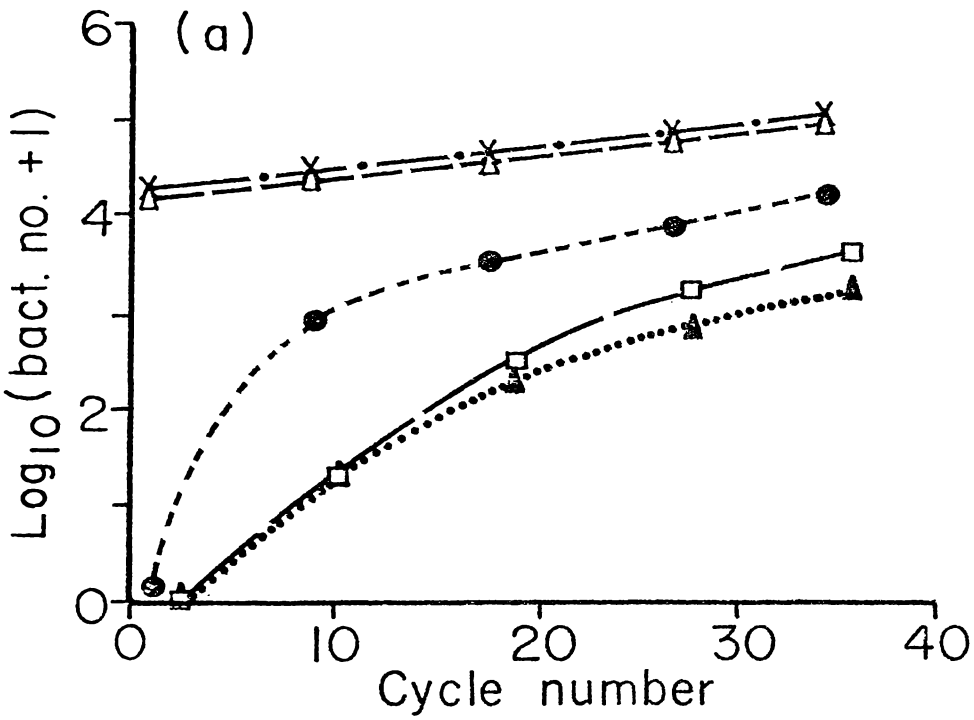


Figure 2.2-3b: Changes in numbers of viable S. faecalis organisms on stainless steel surfaces after components of treatment A.2 (no final rinse) - presented three-dimensionally


(a)
6
4
2
0
Log10 (bact. no. +1)
0
10
20
30
40
Cycle number

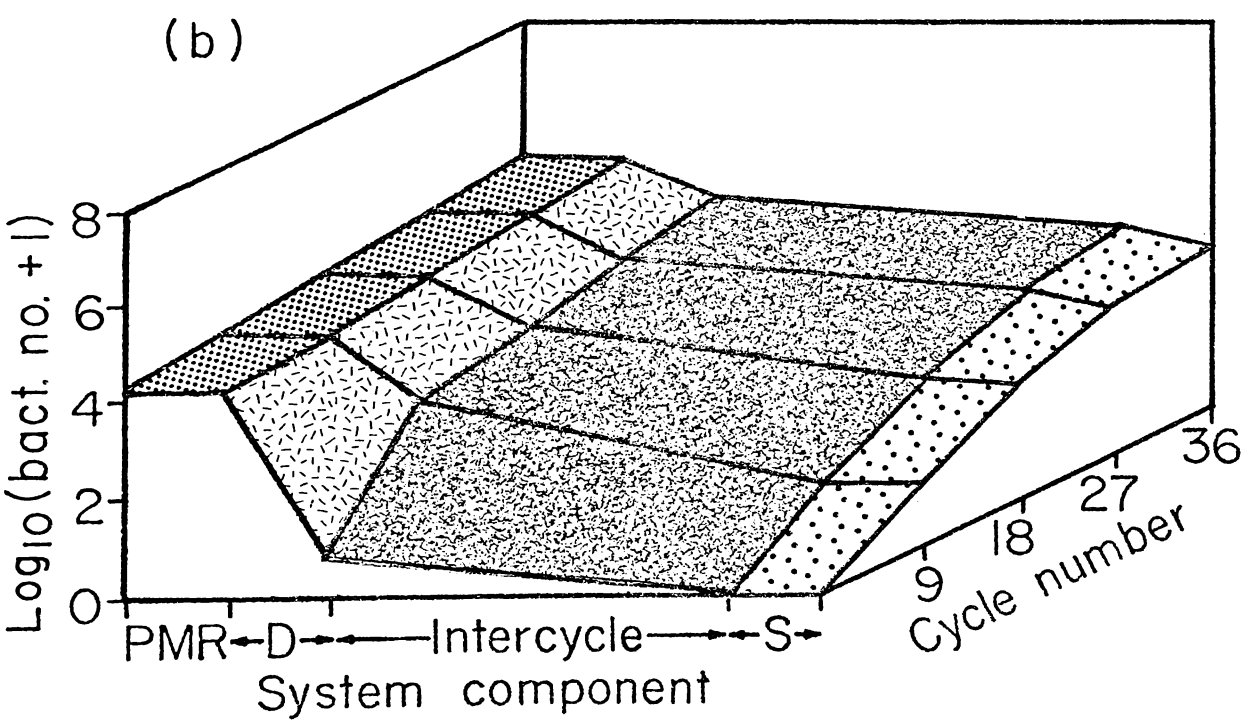

(b)
8
6
4
2
0
Log10 (bact. no. +1)
PMR
D
Intercycle
S
System component
9
18
27
36
Cycle number

Figure 2.2-4a: Changes in numbers of viable S. faecalis organisms on stainless steel surfaces after components of Treatment A.3 (no post-milk rinse)

Key:
1. After milk soiling
2. After detergent
3. After final solution
4. After intercycle period
5. After pre-milk sanitizer

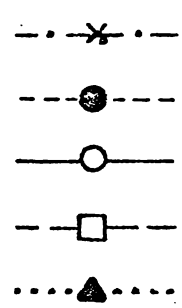
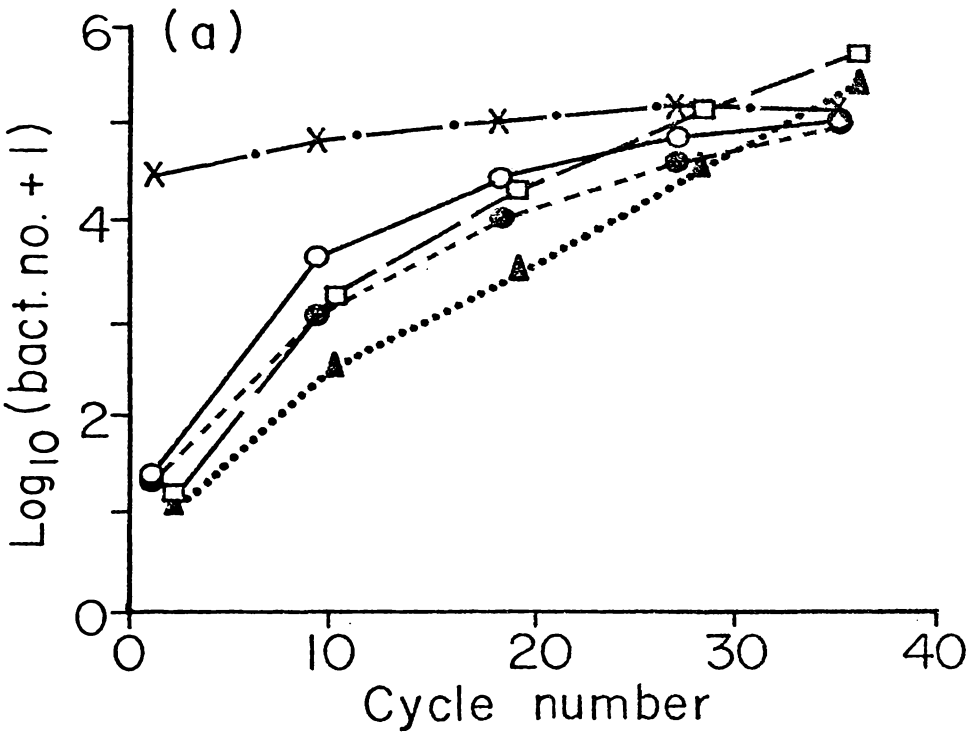


Figure 2.2-4b: Changes in numbers of viable S. faecalis organisms on stainless steel surfaces after components of treatment A.3 (no post-milk rinse) - presented three-dimensionally.


(a)
6
4
2
0
Log10 (bact. no. + 1)
0
10
20
30
40
Cycle number

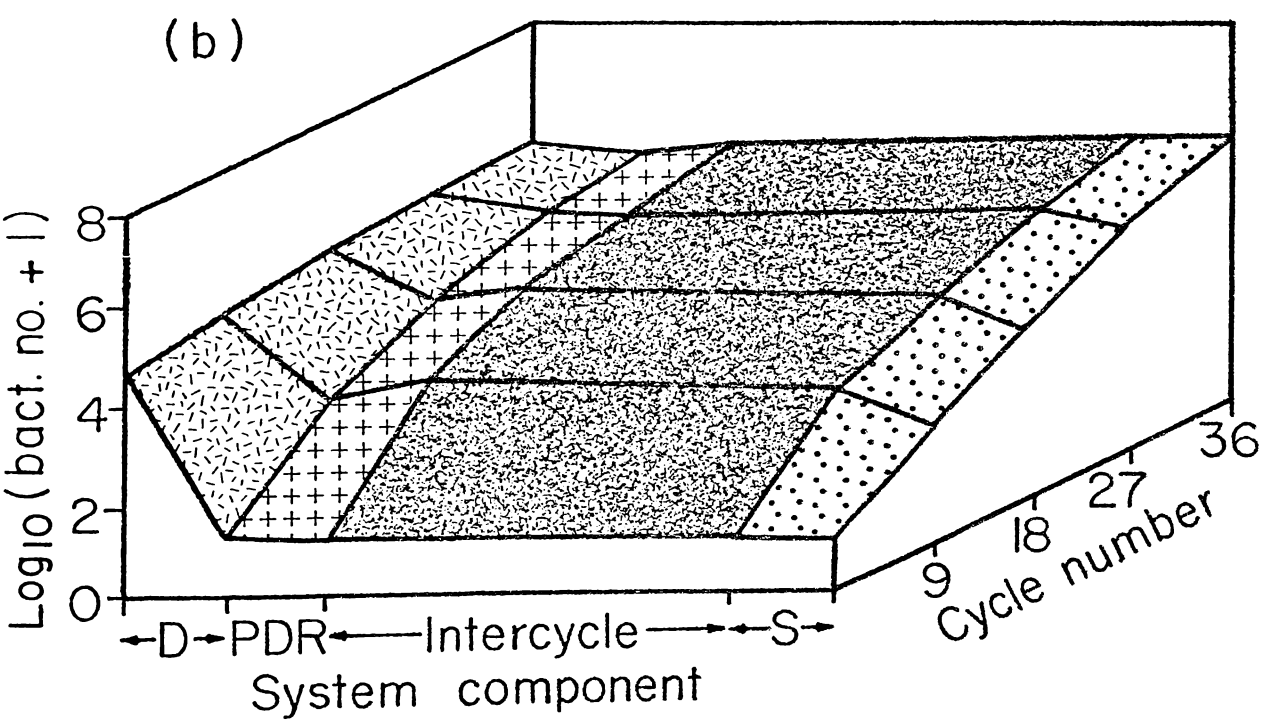

(b)
8
6
4
2
0
Log10 (bact. no. + 1)
←D→PDR←—Intercycle—→←S→
System component
9
18
27
36
Cycle number

Figure 2.2-5a: Changes in numbers of viable S. faecalis organisms on stainless steel surfaces after components of Treatment A.4 (no detergent)

Key:
1. After milk soiling
2. After post-milk rinse
3. After "detergent"
4. After intercycle period
5. After pre-milk sanitizer

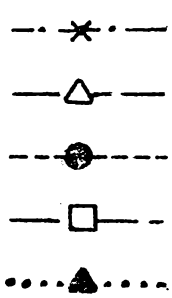
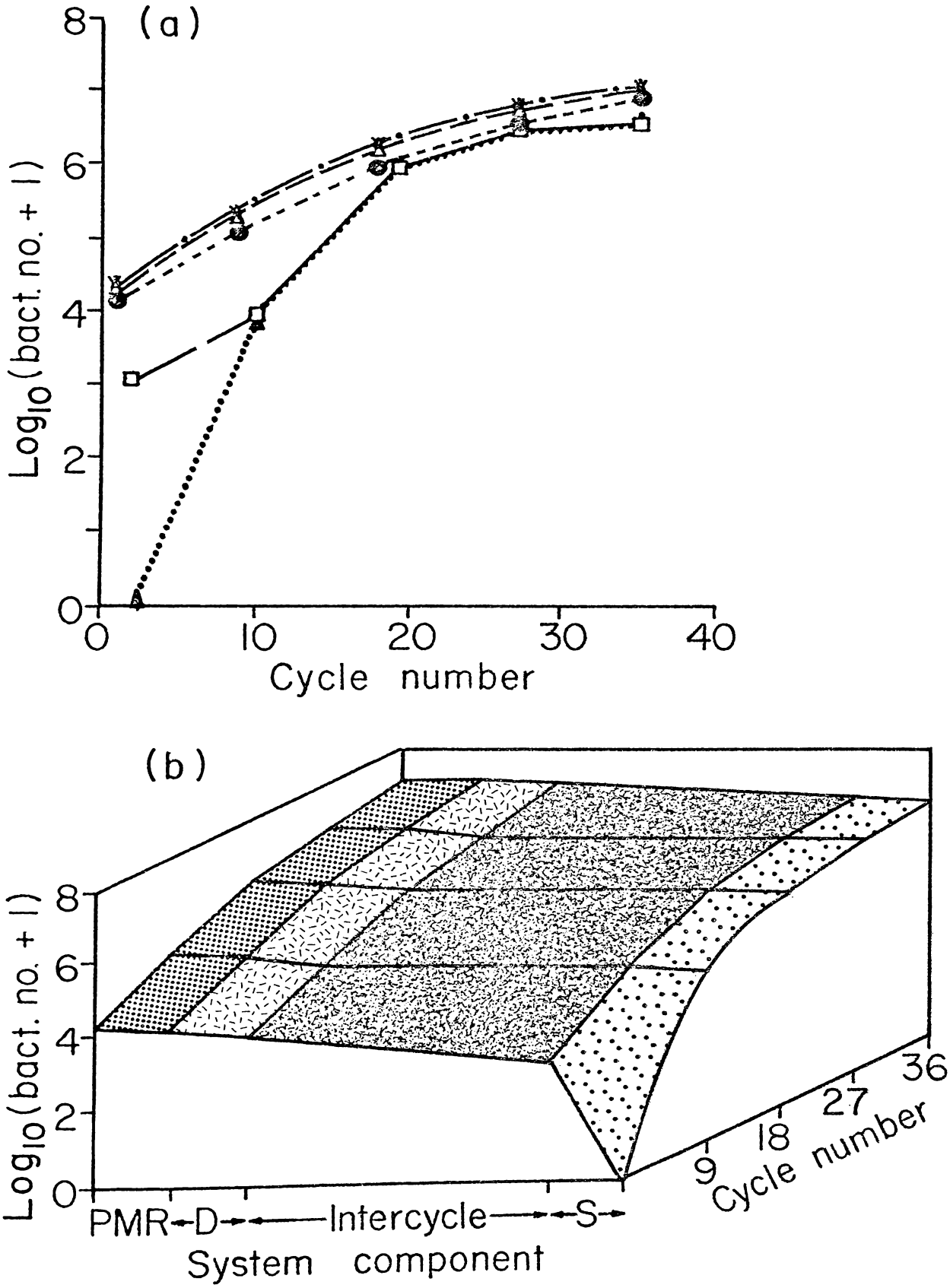


Figure 2.2-5b: Changes in numbers of viable S. faecalis organisms on stainless steel surfaces after components of treatment A.4 (no detergent) - presented three-dimensionally


(a)
8
6
4
2
0
Log10(bact. no. + 1)
0
10
20
30
40
Cycle number
(b)
8
6
4
2
0
Log10(bact. no. + 1)
PMR
D
Intercycle
S
System component
9
18
27
36
Cycle number

post-wash rinse, the intercycle time and the pre-milk sanitizer components of the system respectively, while Figures 2.2-6d, 2.2-7b, 2.2-7d, 2.2-8b and 2.2-8d present the reduction in count caused by these respective components.

The numbers of organisms surviving exposure to the whole system (i.e. after the pre-milk sanitizer) are presented in Figure 2.2-9a and the reduction in count achieved by the systems from after soiling in one cycle, to immediately before soiling in the next cycle are presented in Figure 2.2-9b.

2.2.3 DISCUSSION

PERFORMANCE OF THE TOTAL SYSTEM

The soil mass data (Figure 2.2-1) show that the detergent is the most important system component in terms of effecting control of deposit. The post-wash rinse is the next most effective system component, while the post-milk rinse contributed very little to the total system performance.

In terms of the mass of soil deposited the systems were ranked A.1 < A.3 < A.2 < A.4 (Figure 2.2-1). In terms of the bacterial numbers surviving the total system the systems were ranked A.1 < A.2 < A.3 < A.4 (Figure 2.2-9a). Thus, there is an overall relationship between the soil mass accumulated and the bacteriological control achieved by a system. However, the observation that System A.2 achieved greater bacteriological control than System A.3, despite leaving a greater soil mass, is probably due to the presence of residual detergent chemicals on the surfaces during the intercycle period in System A.2. These chemicals were removed by post-wash rinsing in System A.3.

Figure 2.2-6: Effect of cleaning system on the influence of milk soiling and post-milk rinsing on *S. faecalis* numbers on stainless steel surfaces

(a) Survivors of milk soiling

(b) Count increase with soiling

(b) Survivors of post-milk rinse

(d) Count decrease with post-milk rinse

Key: Treatment A.1 (triple)

Treatment A.2 (no final rinse)

Treatment A.3 (no post-milk rinse)

Treatment A.4 (no detergent)

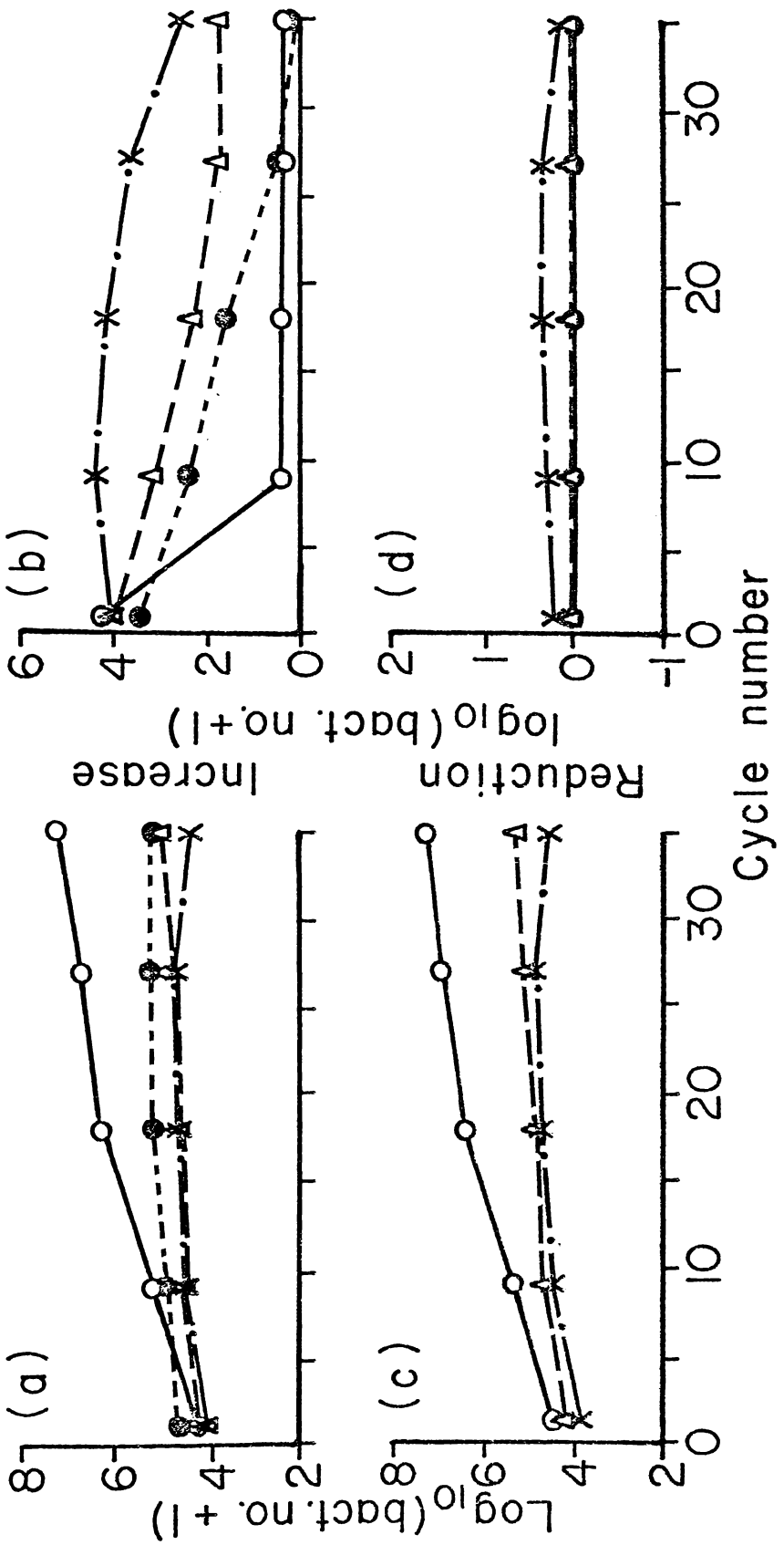

(a)
(b)
(c)
(d)
Log10 (bact. no. + 1)
Increase
Reduction
log10 (bact. no.+1)
Cycle number

Figure 2.2-7: Effect of cleaning system on the influence of detergent washing and post-wash rinsing on S. faecalis numbers on stainless steel surfaces.

(a) Survivors of detergent washing

(b) Count decrease with detergent

(c) Survivors of post-wash rinse

(d) Count decrease with post-wash rinse

Key: Treatment A.1 (triple)

Treatment A.2 (no final rinse)

Treatment A.3 (no post-milk rinse)

Treatment A.4 (no detergent)

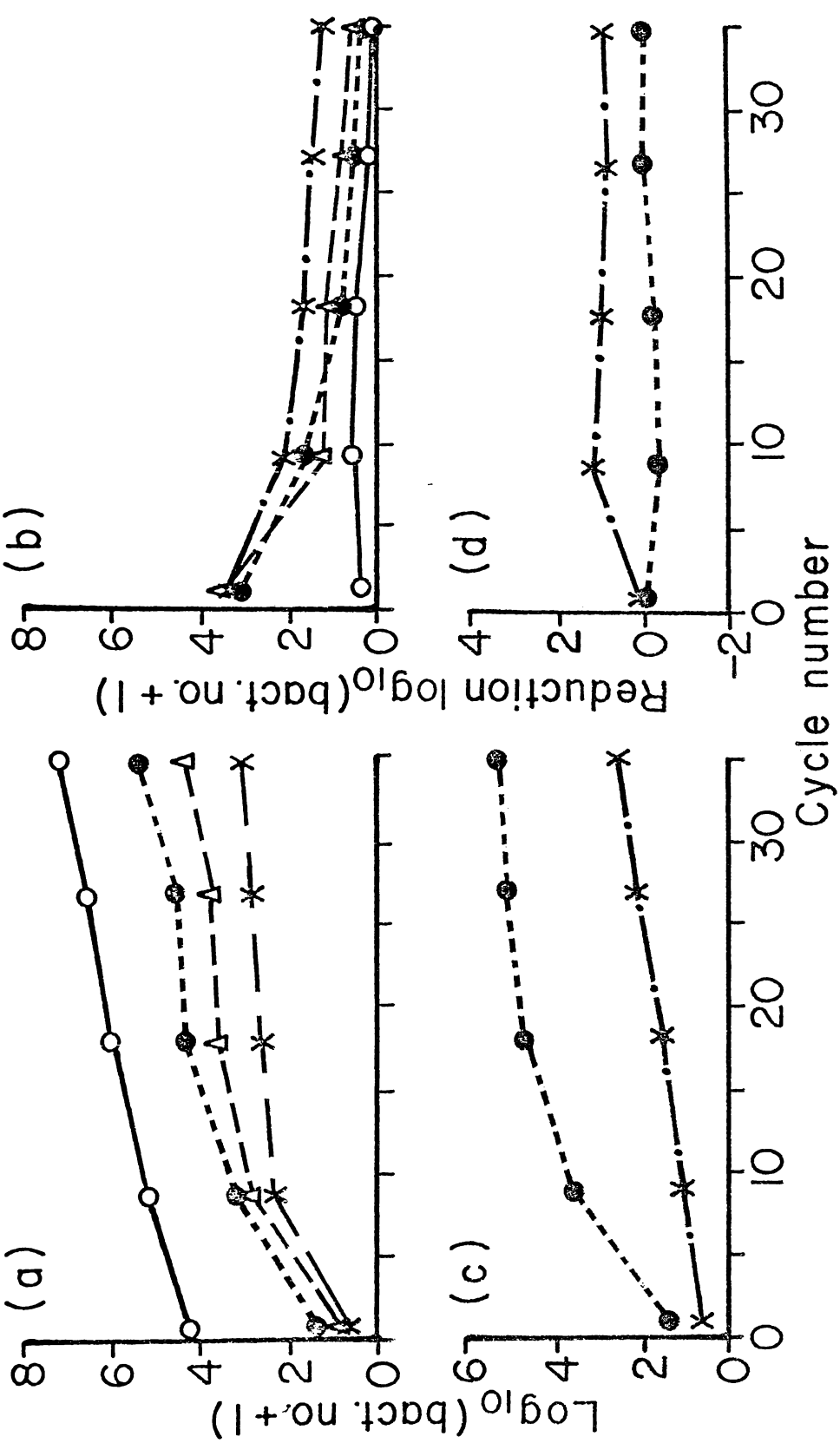

(a)
(b)
(c)
(d)
Log10 (bact. no.+1)
Reduction log10(bact. no.+1)
Cycle number
0
2
4
6
8
10
20
30
-2

Figure 2.2-8: Effect of cleaning system on the influence of intercycle time and pre-milk sanitizing on *S. faecalis* numbers on stainless steel surfaces

(a) Survivors of Intercycle time

(b) Count decrease in intercycle time

(c) Survivors of pre-milk sanitizing

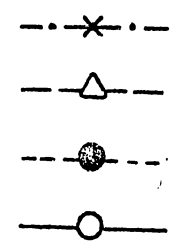
(d) Count decrease with pre-milk sanitizing

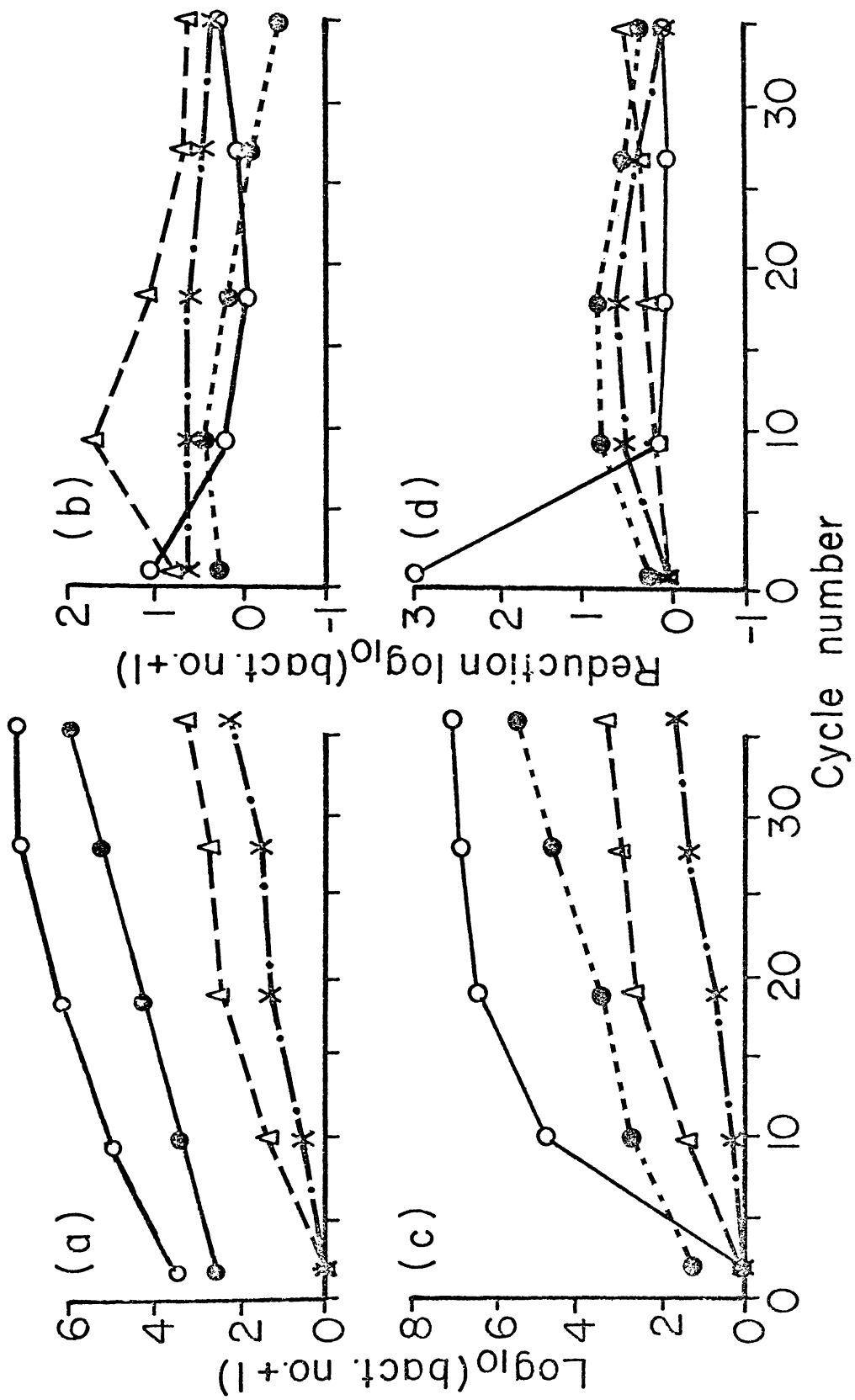
Key: Treatment A.1 (triple)

Treatment A.2 (no final rinse)

Treatment A.3 (no post-milk rinse)

Treatment A.4 (no detergent)




(a)
(b)
(c)
(d)
Log10 (bact. no.+1)
Reduction log10 (bact. no.+1)
Cycle number

In System A.1 (Figure 2.2-2b) the post-milk rinse did not reduce the surface count. The detergent was the single most effective component of the system bacteriologically, but it's effect diminished with time. The post-wash rinse reduced the count by only a small amount. During the intercycle period there was a very slight decrease in numbers of viable S. faecalis. The sanitizer contributed very little to the total system performance.

The system without a post-wash rinse (A.2 — Figure 2.2-3b) showed a similar pattern, except for a greater reduction in count during the intercycle period.

In System A.3 (no post-milk rinse) the detergent performed similarly to the systems A.1 and A.2 but the post-detergent rinse did not reduce the count at all. The sanitizer effect was again only small.

In System A.4 (no detergent) the two water solutions (post-milk rinse and "detergent") had little effect on the numbers of surface S. faecalis and there was little change during the intercycle time. In early cycles when the soil accumulation was low the sanitizer had a considerable effect,as there was a high number of organisms at the end of the intercycle period. This effect quickly diminished, however, as the soil accumulated to high levels, inhibiting the bactericidal effect of the sanitizer.

PERFORMANCE OF SYSTEM COMPONENTS

Milk Soiling

The numbers of S. faecalis organisms on the surfaces after soiling increased slowly as the cycle number increased (Figure 2.2-6a). The numbers were related to the amount of soil on the surfaces; with System A.4 being far greater than Systems A.3, A.2 and A.1. The

Figure 2.2-9 : Influence of 4 complete cleaning systems on the numbers of S. faecalis organisms on stainless steel surfaces.

(a) Survivors of cleaning systems

Key: Treatment A.1 (triple)

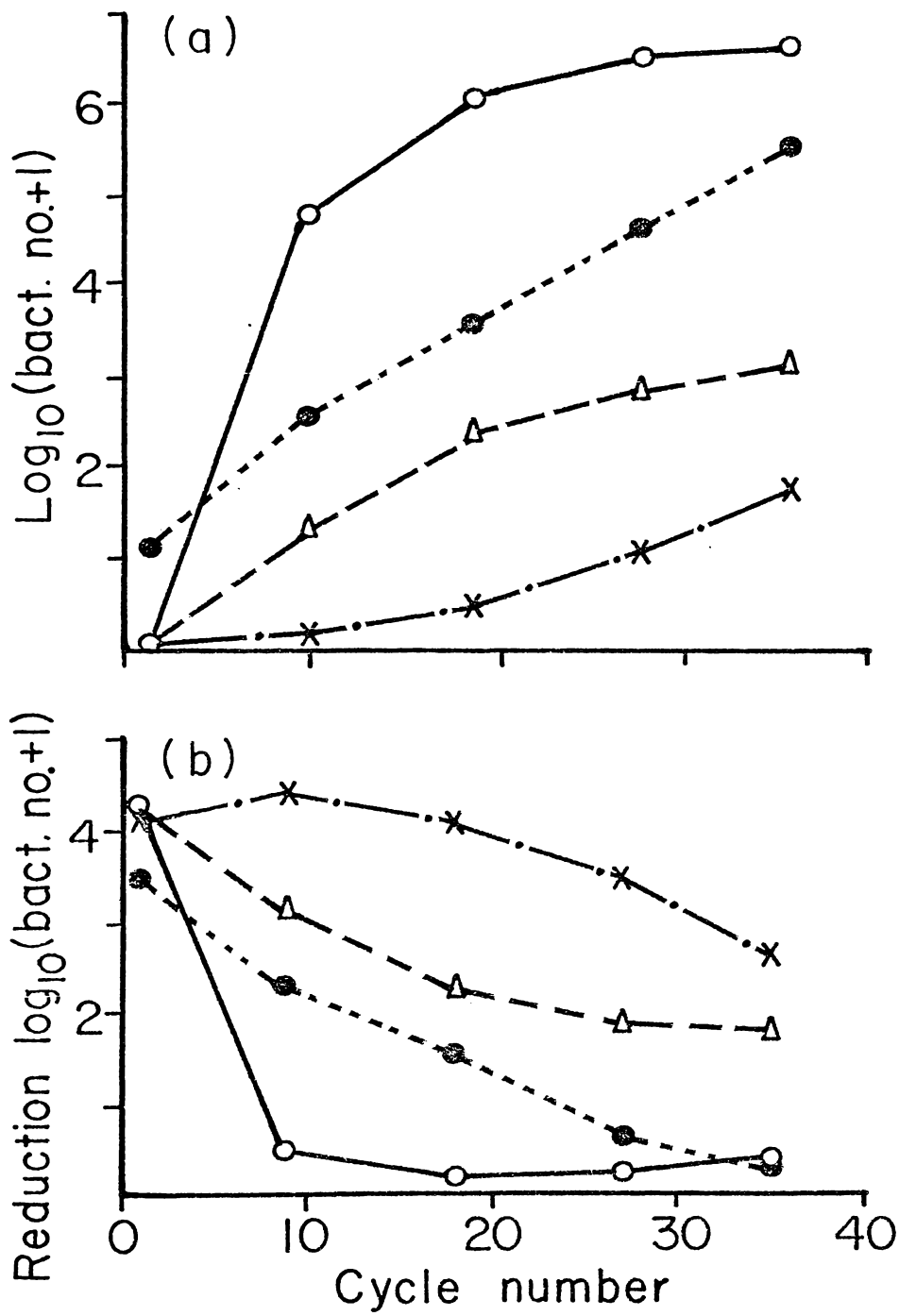
Treatment A.2 (no final rinse)

Treatment A.3 (no post-milk rinse)

Treatment A.4 (no detergent)

(b) Count decrease with cleaning systems

Key: as for 2.2-9a.


(a)
6
4
2
Log10(bact. no.+1)
(b)
4
2
Reduction log10(bact. no.+1)
0
10
20
30
40
Cycle number

numbers adhering at any time varied with the amount of soil on the surface (Figure 2.2-6b). As the numbers on the surfaces before soiling increased, while the numbers after soiling changed little over time, there was a decrease in the numbers attaching over time. The systems with poorest bacterial control (A.3, A.4) reached a stage in the later cycles where a steady state was established, with equal numbers of organisms being laid down in the soil and being removed from the surface by the soiling solution.

Effect of Post-Milk Rinse

The post-milk rinse had a neglibible effect on the surface counts (Figure 2.2-6d). As a result,the numbers of survivors after the post-milk rinse were the same as those after the milk soiling (Figure 2.2-6c).

Effect of Detergent

The reduction in count achieved by the detergent (Figure 2.2-7b) showed it to be the single most effective component of the system in terms of reducing the numbers of surface organisms. The count reduction achieved was dependent upon three factors:

1. The efficiency of the detergent mixture used; the only difference between Systems A.2 and A.4 was the addition of detergent chemicals to the wash solution in System A.2.
2. The use of a post-milk rinse; the detergent in system A.3, which was not preceded by a post-milk rinse, was less efficient than that in system A.2 in which the detergent followed such a rinse.
3. The use of a post-wash rinse; the use of a post-wash rinse in System A.1 improved the removal of the detergent/soil mixture which remained after the washing operation. This permited this

system to perform more efficiently than System A.2 which lacked this post-wash rinse.

Effect of Post-Wash Rinse

Like the post-milk rinse, the post-wash rinse had very little effect upon the numbers of surface organisms (Figures 2.2-7c and 2.2-7d).

Effect of Intercycle Period

In all treatments the intercycle conditions were inappropriate to support significant growth of S. faecalis during the intercycle period (Figure 2.2-8b). The reduction in count was greater in System A.2, as the residual detergent chemicals were left in contact with the surface during the intercycle period and had an inhibitory effect on the bacteria for that time. In Systems A.3 and A.4 at high cycle numbers, where the soil load was comparatively greater, there was no significant change in the numbers of S. faecalis during the intercycle period.

Effect of Pre-Milk Sanitizer

The same pre-milking iodophor sanitizer was applied in all four systems. However, the sanitizer effect varied with the numbers available at the start of the sanitizing operation and also the amount of soil in the substrate (Figure 2.2-8b). The difference in the reduction in count between Systems A.3 and A.4 (Figure 2.2-8d), which had similar surface numbers before pre-milk sanitizing (Figure 2.2-8a), may result from the differing interaction between sanitizer and soil. The soil in System A.3 had been washed with detergent, while that in A.4 merely water rinsed - therefore they were qual-

itatively different.

THE MICRO-ECOLOGY OF THE SURFACES

In the conditions of this experiment the S. faecalis organisms acted in many circumstances as inert particles. The changes in their numbers have merely reflected the changes a component of the milk soil would experience. There was no evidence for growth on the surfaces, nor did the data show that the S. faecalis was participating in any succession of organisms in the community on the surfaces of the slides.

The S. faecalis organisms were being deposited onto the surfaces in constant numbers in the milk soil. It is not known whether they were attaching directly to the surface of the substrate or to milk components which were themselves attached. Had sufficient water remained available this environment probably would have provided favourable growth conditions for the organisms. The fact that there was not sufficient water (relative humidity 80%) is shown by the absence of growth during the intercycle period in System A.4 in which no detergent was applied.

In the other systems (A.1, A.2, A.3) most of the milk soil, and the bacteria it contained was removed by the detergent wash. A few of the bacteria were killed by the sanitizing treatment, but probably only those exposed at the surface of the soil, and not the majority which were protected by the soil.

SUMMARY

The major results of this experiment were:

1. Of all the system components, the detergent contributed most to bacterial control, with a smaller contribution by the sanitizer;

2. The numbers of organisms on the surface were related to the amount of soil on the surface (detergent efficiency), but this relationship was altered by other factors (e.g. residual chemicals on surfaces during the intercycle time);
3. The changes in cleaning system with time were documented;
4. The intercycle conditions of milk soil (a temperature of 30°C and a relative humidity of 80%) were not satisfactory for the growth of S. faecalis during the 9.5h intercycle period;
5. The simulator technique was shown to be a satisfactory method of analysing the performance of cleaning systems.

2.3 SIMULATOR INVESTIGATION OF THE TIMING OF SANITIZING

In the first experiment the influence of the detergent on the cleaning performance of the total system was studied. The second experiment deals with the role of the sanitizer. Sanitizers are traditionally applied after the detergent, but in milking machines this sanitizer can be applied as a post-wash rinse at the start of the intercycle period, or as a pre-milk rinse at the completion of the intercycle period. The pre-milking application has achieved superior bacteriological control in the field (Middleton et al. 1965) but often requires a following water rinse to prevent sanitizer residues entering the milk. It is, however, the only cleaning task which is necessary prior to milking and is therefore an operation which many dairymen find onerous. Although the post-wash sanitizer is slightly less effective in bacterial control, the long drying time involved often overcomes the need for a water rinse (Dunsmore et al. 1977a). Also the sanitizing is performed as one task in the total post-milk washing program, and is therefore more acceptable to the dairyman.

As the efficiency of both detergency and sanitizing are markedly improved by increasing temperature, attempts to clean milking machines with systems comprised of cold solutions places limits upon the performance of the system. It was possible that increasing the contact-time of cold detergents and sanitizers could overcome some of the disadvantages of this system. With milking machines the intercycle period offers the opportunity to have a solution contact with the surface for $\sim$ 10h, and thus change the microenvironment considerably.

The aims of the experiment described here were to investigate:

1. The contribution of the sanitizer to the performance of the total system;
2. The relative performance of pre-milk and post-wash sanitizers; and
3. The bacterial control achieved by a sanitizer applied for the complete intercycle period.

2.3.1 EXPERIMENTAL DESIGN

The four treatments of the experiment are presented in Table 2.3-1. System B.1 (no sanitizer) consisted of four steps; a pre-milking rinse of cold water, alkaline detergent applied immediately after the milk, a post-detergent rinse of cold water and a final solution of cold water. In System B.2 (pre-milk sanitizer) an iodophor solution was substituted for the pre-milk water rinse of System B.1. In System B.3 (post-wash sanitizer) an iodophor solution was substituted for the final water solution of system B.1. In System B.4 (intercycle sanitizer) the iodophor was again applied as the final solution, but remained in contact with the slides until drained away immediately before the next milk soiling, before which no pre-milk solution was applied. The alkaline detergent (3g/l) and iodophor (1.5 ml/l) were the same as those used in the previous experiment.

The experimental treatments were applied to the stainless steel slides for 28 cycles, with samplings performed at cycles 3/4, 9/10, 15/16, 21/22 and 27/28. At each sampling, quadruplicate samples were taken for surface bacterial counts and gravimetric assessment of soil at the times shown in Table 2.3-1.

Table 2.3-1: Cleaning systems applied and sampling times in study of timing of sanitizing

Cleaning system		Components of cleaning systems					
No.	Name	Pre-milk	Milk	Alk. Detgt 50°C	Water rinse 20°C	Final soln	Inter-cycle
B.1	No sanitizer	Water 20°C	+	+	+	Water 20°C	-
B.2	Pre-milk sanitizer	Iodophor 25 mg Av. I/1, 20°C	+	+	+	Water 20°C	-
B.3	Post-wash sanitizer	Water 20°C	+	+	+	Iodo-phor 25mg Av.I/1 20°C	-
B.4	Intercycle sanitizer		+	+	+	Iodo-phor 25mg Av.I/1 20°C	
Sampling times *		I	S, G	M	R	F	

* I bacterial count of surfaces after intercycle period (before pre-milk solution),
S bacterial count of surfaces after pre-milk soln. (before milk soiling),
M bacterial count of surfaces after milk soiling (before detergent),
R bacterial count of surfaces after detergent wash and post-detergent rinse (before final solution),
F bacterial count after final solution (before intercycle period),
G Gravimetric assessment of soil after pre-milk solution (before milk soiling).

The test cells were maintained at a temperature of 30°C and the relative humidity at 85%. The dosing organisms used were S. faecalis and E. aerogenes, both being added to the soiling milk at a rate of 5×10^5/ml. The iodophor and alkaline detergents were sterile, but the water rinses were contaminated with an organism resembling E. aerogenes as the filter system on the water supply broke down.

2.3.2 RESULTS

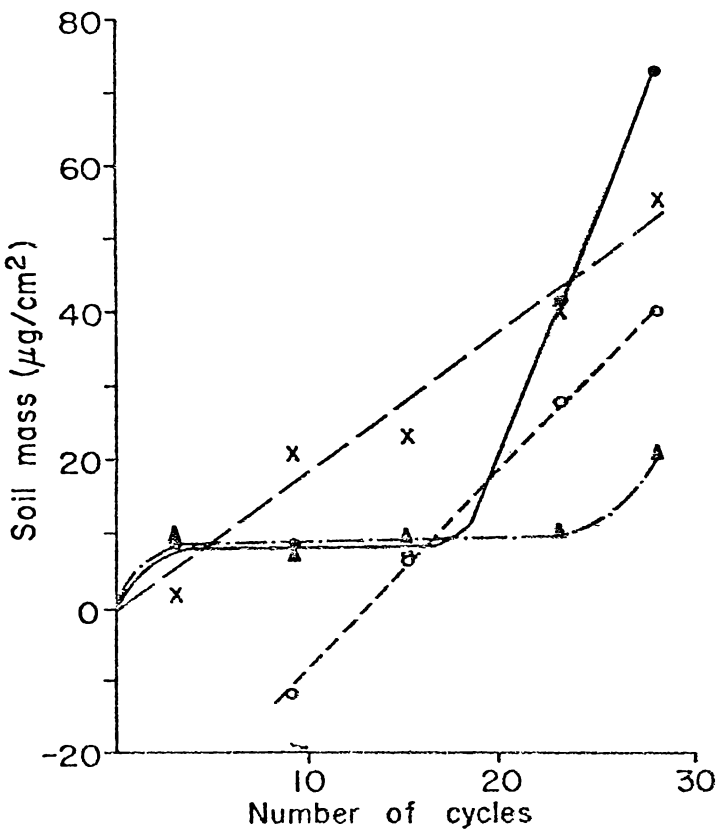
The changes in soil mass on the slide surfaces with the four treatments are presented in Figure 2.3-1. This shows that the two systems in which iodophor was in contact with the slides immediately before milk soiling (Systems B.2 and B.4) had a three-part accumulation curve: a small initial increase to a plateau level at low deposit, and later a high rate of soil accumulation. The two systems without the pre-milk iodophor (Systems B.1 and B.3) both had a linear rate of soil increase. The application of iodophor in any way, whether pre-milk, post-wash or intercycle (Systems B.2, B.3 and B.4) reduced the amount of soil accumulation, at least until cycle 25, compared to the no-sanitizer treatment (System B.1).

The changes in the numbers of viable S. faecalis on surfaces at the various stages of the cleaning systems over 28 cycles are presented in three-dimensional form in Figures 2.2-2a, -3a, -4a and-5a. The similar data for E. aerogenes are presented in Figures 2.2-2b, -3b, -4b and -5b.

The influence of system components upon the numbers of S. faecalis and E. aerogenes on the slide surfaces is shown in Figures 2.3-6 (milk soiling), 2.3-7 (detergent and rinse), 2.3-8 (final

Figure 2.3-1: Changes in soil mass on stainless steel surfaces over 28 cycles with four cleaning systems in sanitizer timing study

Key:
1. System B.1 (no sanitizer)
2. System B.2 (pre-milk sanitizer)
3. System B.3 (post-wash sanitizer)
4. System B.4 (Intercycle sanitizer)


80
60
40
20
0
-20
Soil mass (μg/cm²)
10
20
30
Number of cycles

solution), 2.3-9 (intercycle time) and 2.3-10 (pre-milk solution). The effects on the numbers of S. faecalis and E. coli of the three application methods of the iodophor sanitizer solution are presented in Figure 2.3-11. Similar data for the four total systems are presented in Figure 2.3-12. In each of these figures two types of information have been presented; the numbers of survivors after the component (or system) has been applied, and also the change in numbers achieved by the component (or system).

The total numbers of organisms, and the relative numbers of S. faecalis and E. aerogenes at each stage of System B.1 (no sanitizer) are presented in Figure 2.3-13. This shows the numbers after milk soiling (a), after detergent-washing and water-rinsing (b), after the final solution (c), after the intercycle period (d) and after pre-milk rinsing (e). It can be seen that in early cycles S. faecalis was the dominant organism in the microflora, while in later cycles, E. coli achieved similar numbers. Most of the differences between the organisms after the detergent and rinse (b), final solution (c), intercycle period (d) and pre-milk solution (e) merely reflected the difference in the relative numbers of the organisms after the milk soiling (a). The changes in total count, and numbers of S. faecalis and E. coli on surfaces cleaned by Systems B.2, B.3 and B.4 followed similar trends to those presented for System B.1.

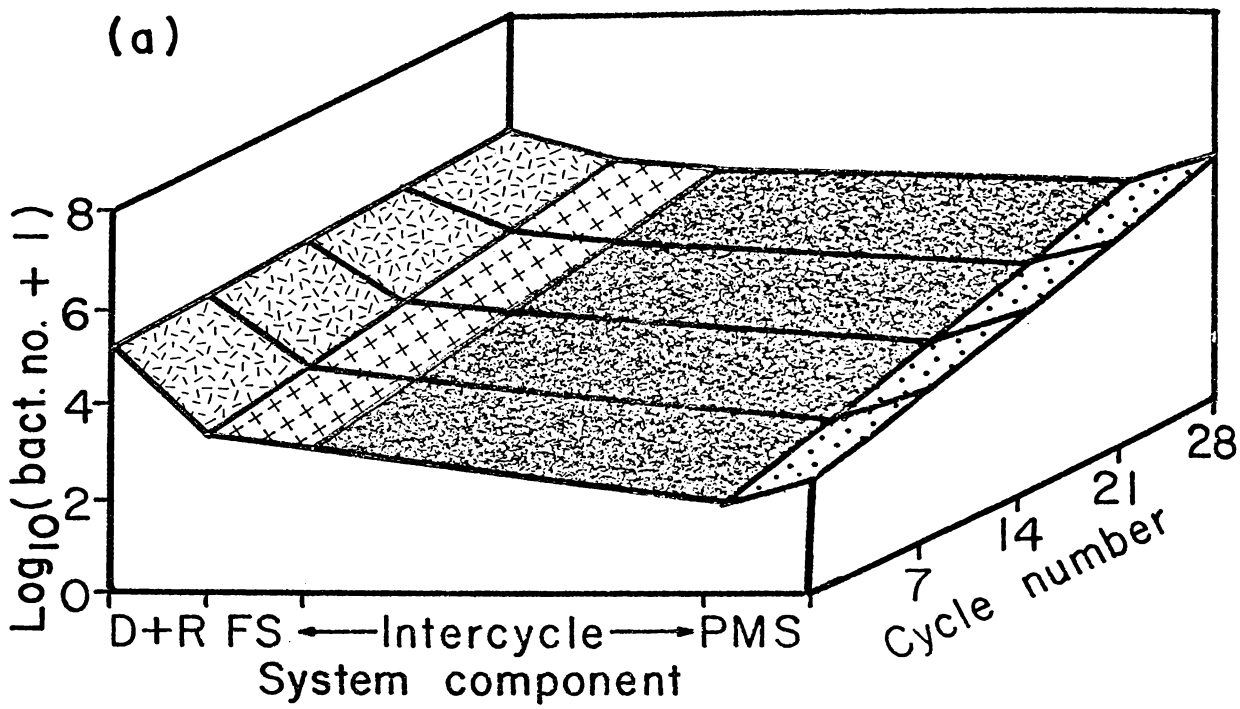
2.3.3 DISCUSSION

TOTAL SYSTEM PERFORMANCE

The soil mass data (Figure 2.3-1) show that the addition of an iodophor improved the detergency of the system by reducing the soil

Figure 2.3-2a: Changes in the numbers of S. faecalis on stainless steel surfaces by components of System B.1 (no sanitizer).

Figure 2.3-2b: Changes in the numbers of E. aerogenes on stainless steel surfaces by components of System B.1 (no sanitizer)


(a)
Log_{10}(bact. no. + 1)
0
2
4
6
8
D+R FS
Intercycle
PMS
System component
7
14
21
28
Cycle number

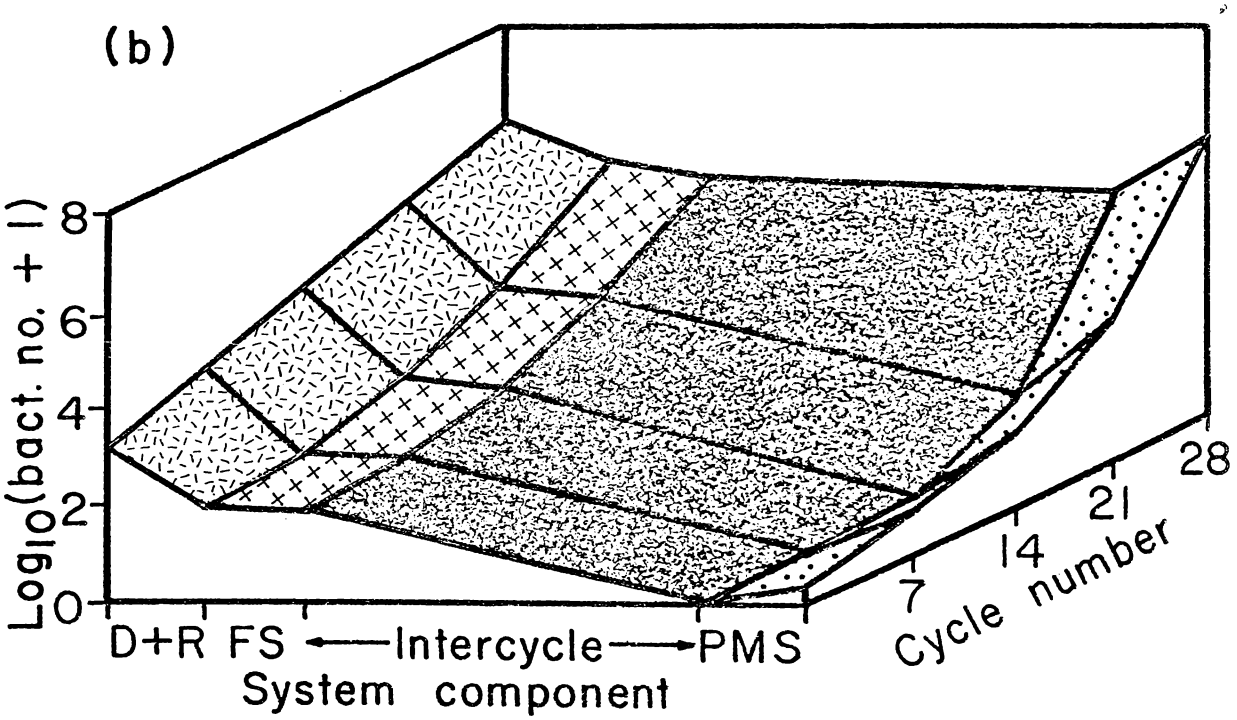
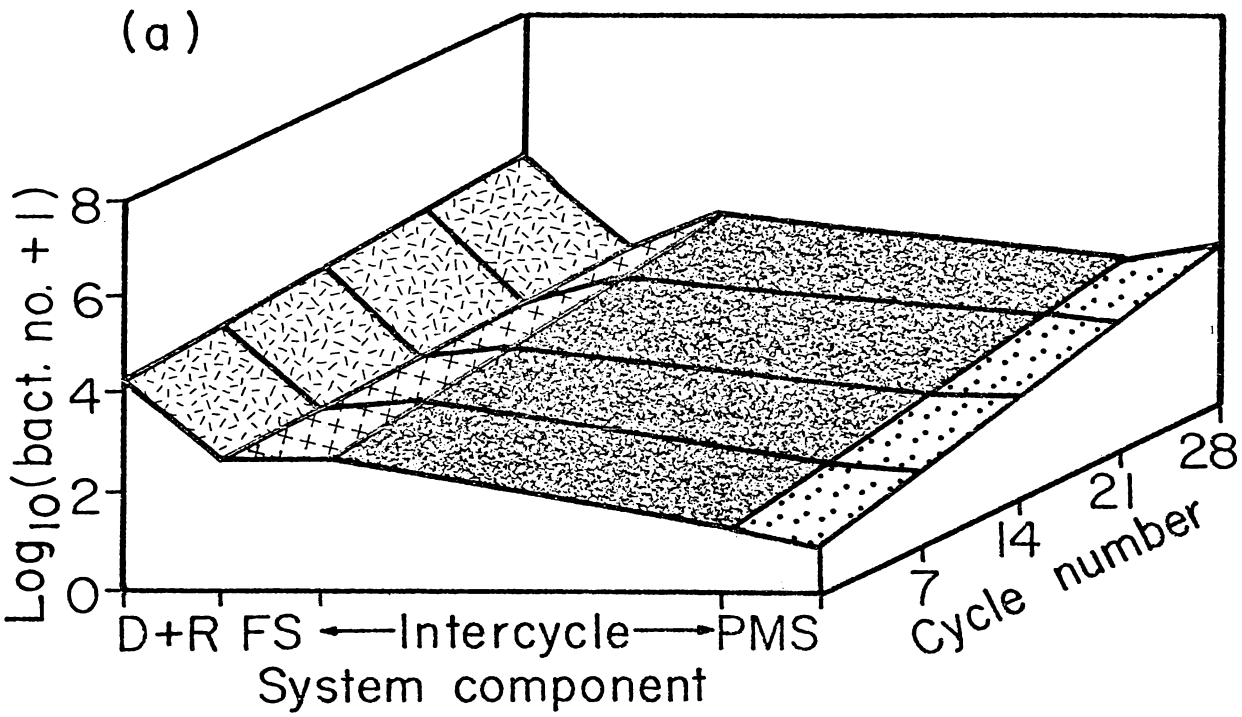

(b)
Log_{10}(bact. no. + 1)
0
2
4
6
8
D+R FS
Intercycle
PMS
System component
7
14
21
28
Cycle number

Figure 2.3-3a: Changes in the numbers of S. faecalis on stainless steel surfaces by components of System B.2 (pre-milk sanitizer).

Figure 2.3-3b: Changes in the numbers of E. aerogenes on stainless steel surfaces by components of System B.2 (pre-milk sanitizer).


(a)
Log10(bact. no. + 1)
8
6
4
2
0
D+R FS
Intercycle
PMS
System component
7
14
21
28
Cycle number

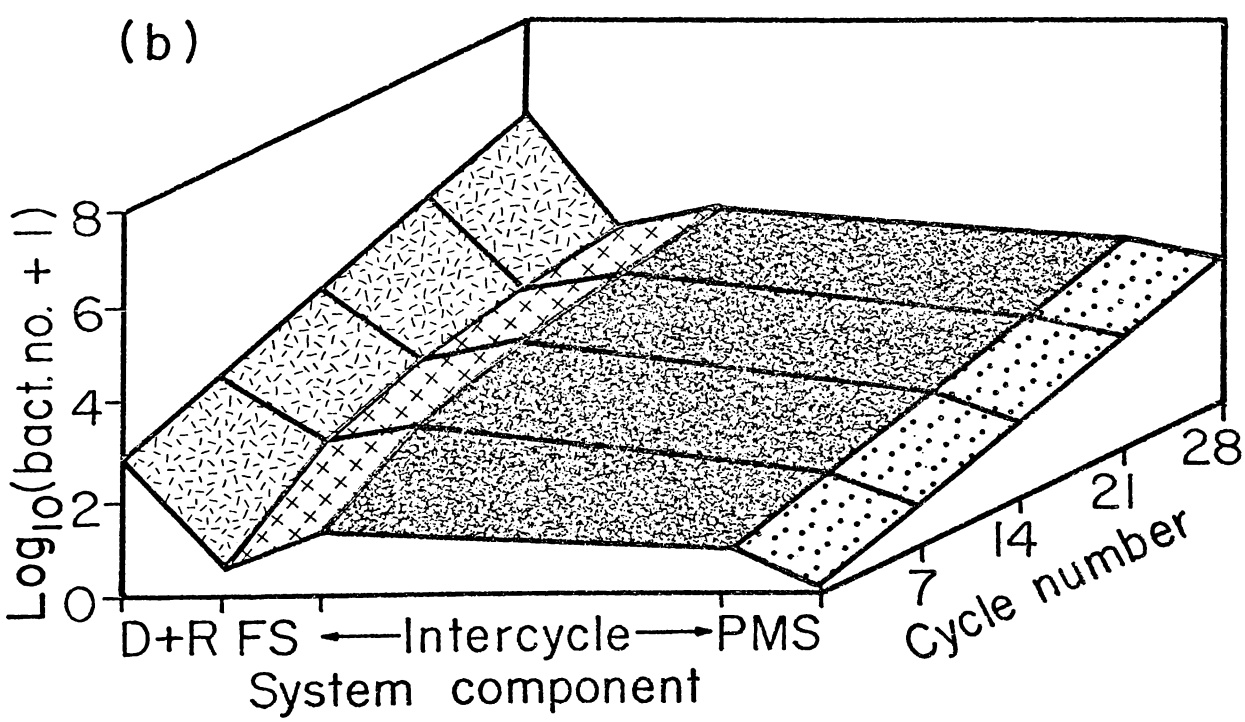
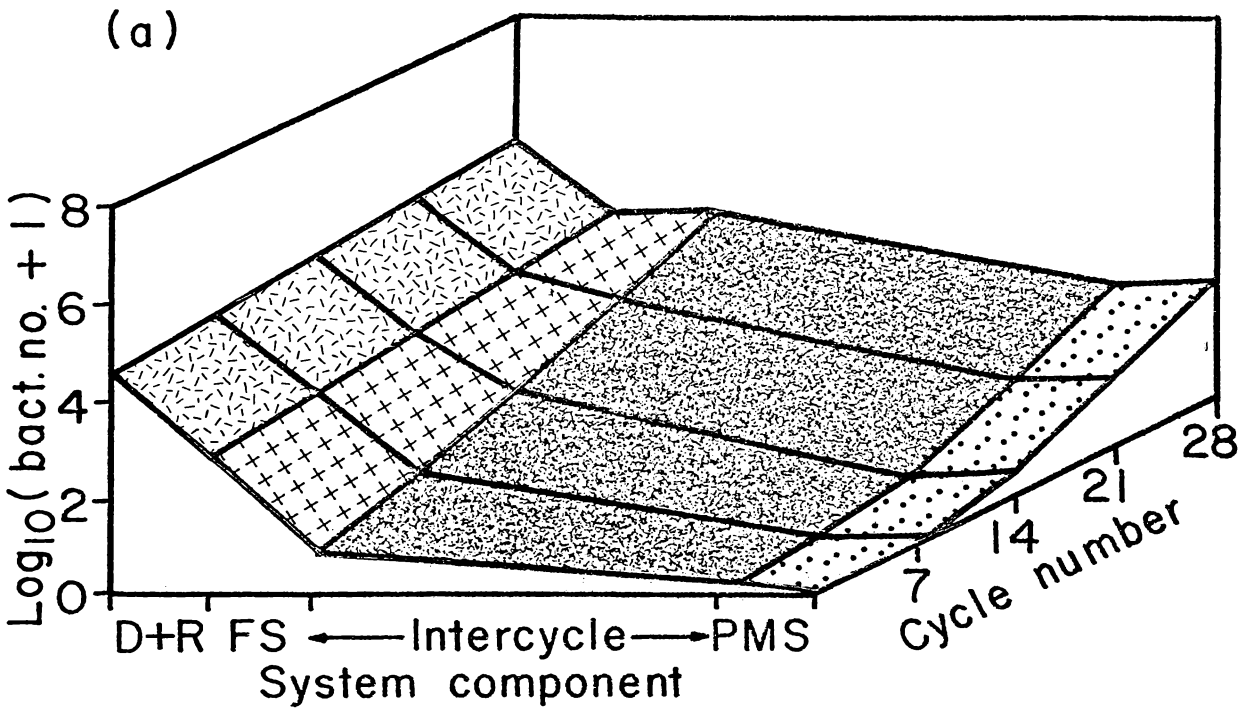

(b)
Log10(bact. no. + 1)
8
6
4
2
0
D+R FS
Intercycle
PMS
System component
7
14
21
28
Cycle number

Figure 2.3-4a: Changes in the numbers of S. faecalis on stainless steel surfaces by components of System B.3 (post-wash sanitizer).

Figure 2.3-4b: Changes in the numbers of E. aerogenes on stainless steel surfaces by components of System B.3 (post-wash sanitizer).


(a)
Log10(bact. no. + 1)
0
2
4
6
8
D+R FS
Intercycle
PMS
System component
7
14
21
28
Cycle number

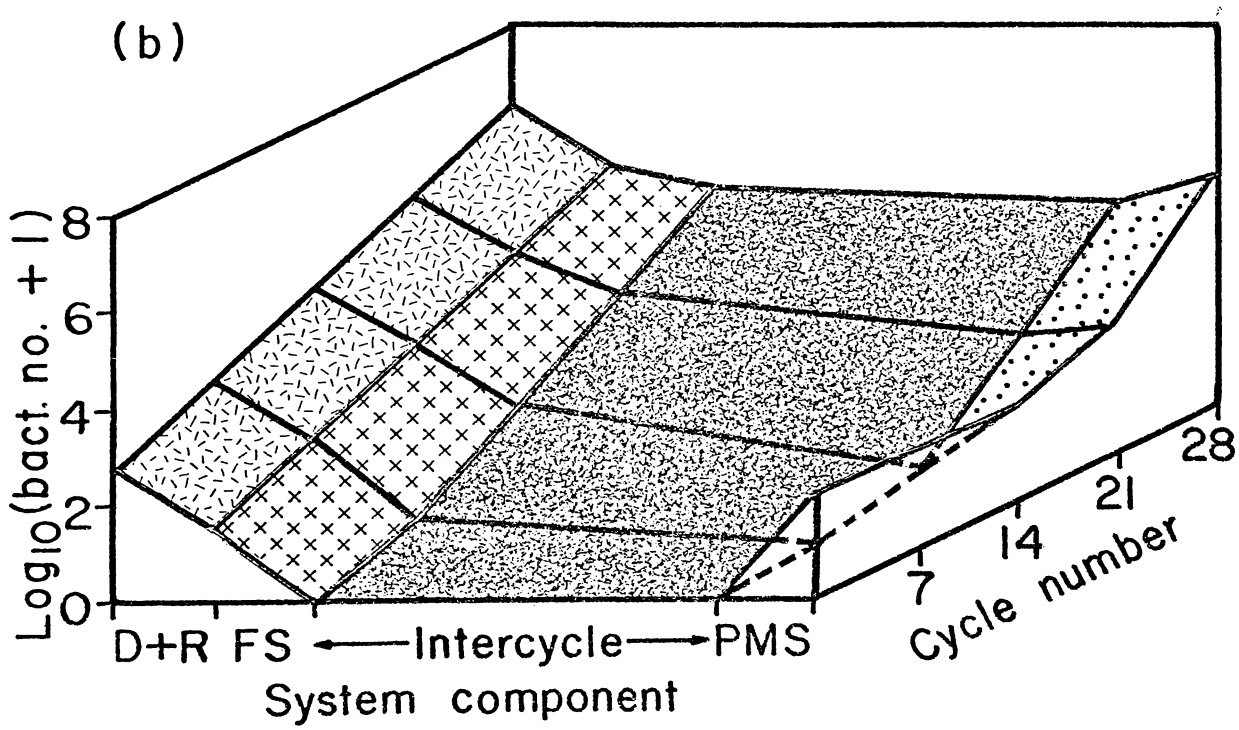
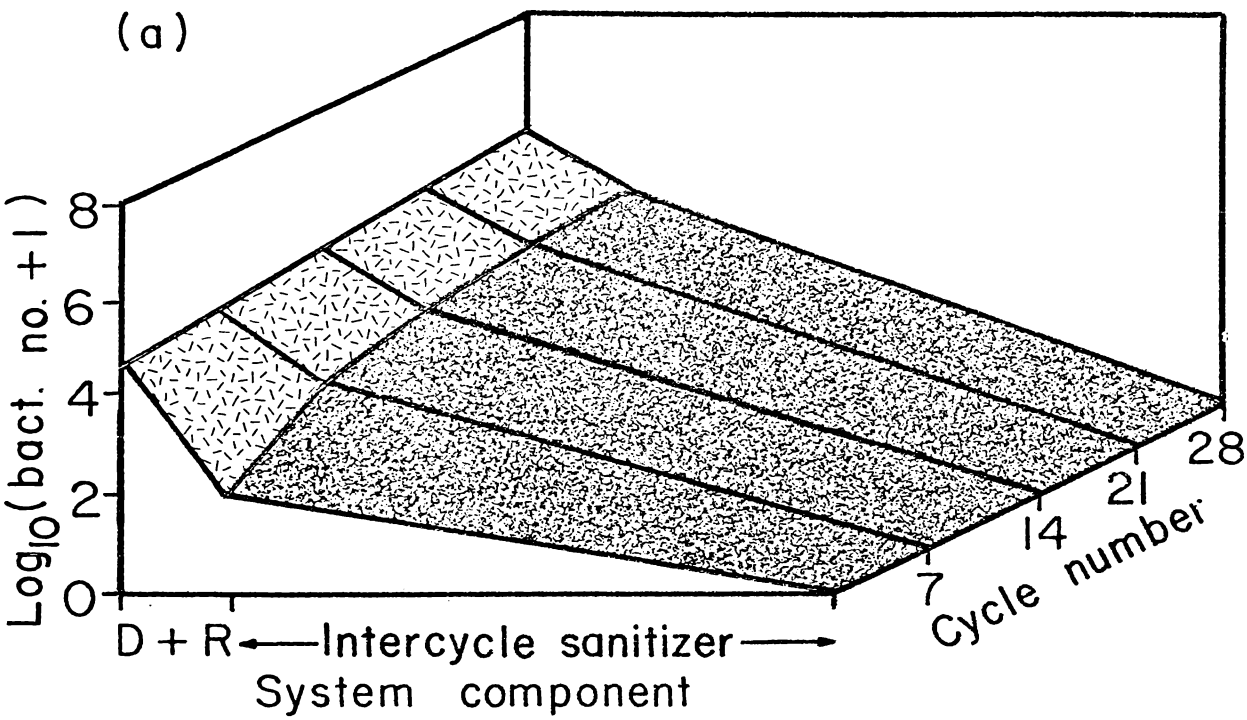
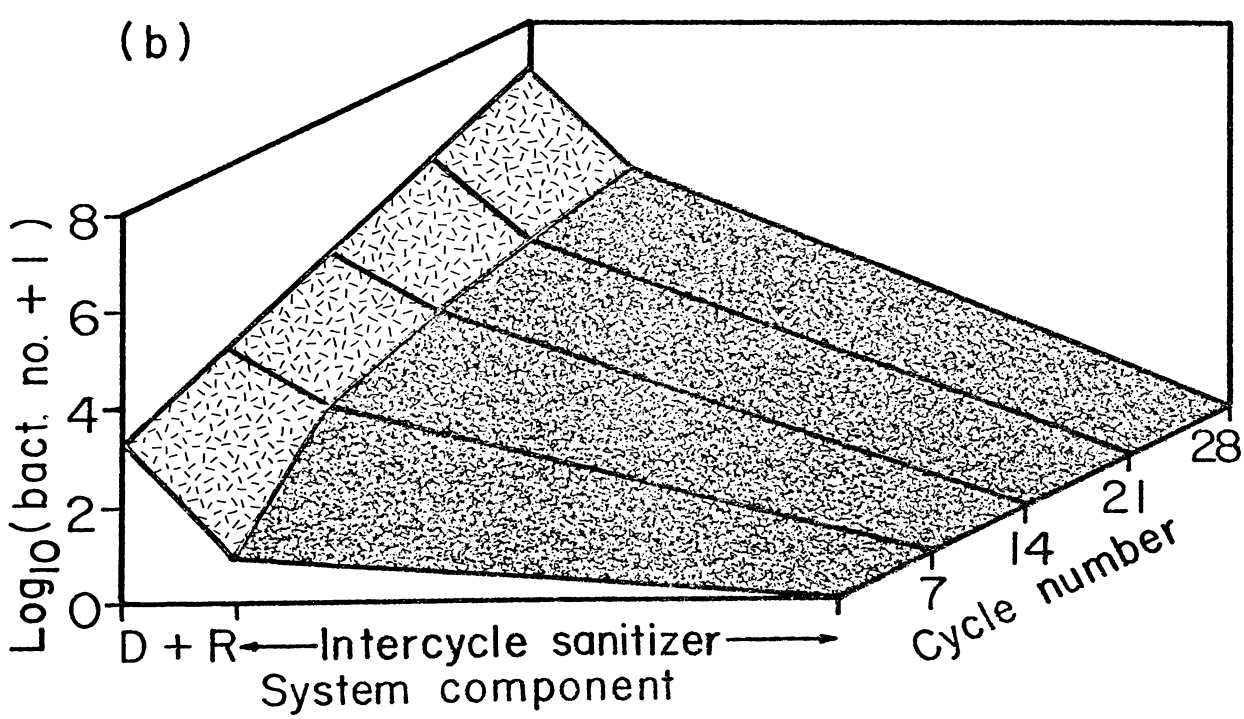

(b)
Log10(bact. no. + 1)
0
2
4
6
8
D+R FS
Intercycle
PMS
System component
7
14
21
28
Cycle number

Figure 2.3-5a: Changes in the numbers of S. faecalis on stainless steel surfaces by components of System B.4 (intercycle sanitizer)

Figure 2.3-5b: Changes in the numbers of E. aerogenes on stainless steel surfaces by components of System B.4 (intercycle sanitizer)


(a)
Log_{10}(bact. no. + 1)
0
2
4
6
8
D + R
Intercycle sanitizer
System component
7
14
21
28
Cycle number


(b)
Log_{10}(bact. no. + 1)
0
2
4
6
8
D + R
Intercycle sanitizer
System component
7
14
21
28
Cycle number

accumulated. The effect of the iodophor differed with its timing within the system. The similarity in soil accumulation characteristics between those systems where the iodophor was the final pre-milk solution suggested that the residues of the iodophor on the substrate surfaces affected the deposition of the milk soil.

In terms of soil mass the systems were ranked B.2 < B.3 < B.1 < B.4 at the end of the experimental period (Figure 2.3-1). In terms of bacterial numbers the systems were ranked B.4 < B.3 < B.2 < B.1 (Figure 2.3-12a). Thus the bacteriological performance of the system is not related to its detergency, but in fact is related to the relative efficiency of the sanitizer used in the system (Figures 2.3-11b and 2.3-11d).

System B-1 (no sanitizer) had similar effects on the numbers of S. faecalis (Fig. 2.3-1a) and E. aerogenes (Fig. 2.3-2b). The detergent and rinse reduced the count significantly after the milk soiling, but the magnitude of the effect diminished with increasing cycle numbers. The water used as the final solution had no effect on the bacterial numbers. In early cycles when soil deposit was low the S. faecalis count reduced by approximately 1 log during the intercycle time, but at 28 cycles, the decrease was negligible, and if the experiment had been continued intercycle growth may have occurred. The drop in numbers of E. aerogenes was much greater than S. faecalis in the early cycles, but these also were changing little during the intercycle period at the end of the experimental period. The pre-milk rinse had little effect on S. faecalis but showed a significant increase with E. aerogenes due to contaminated rinse water.

The detergent was again the most effective component of System

Figure 2.3-6: Influence of cleaning system on milk soil effect on bacterial numbers on surfaces

(a) S. faecalis surviving milk soil

(b) Increase in S. faecalis with milk soiling

(c) E. aerogenes surviving milk soil

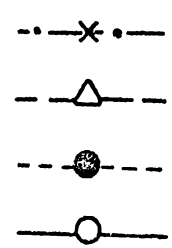
(d) Increase in E. aerogenes with milk soiling

Key: System B.1 (no sanitizer)

System B.2 (pre-milk sanitizer)

System B.3 (post-wash sanitizer)

System B.4 (intercycle sanitizer)



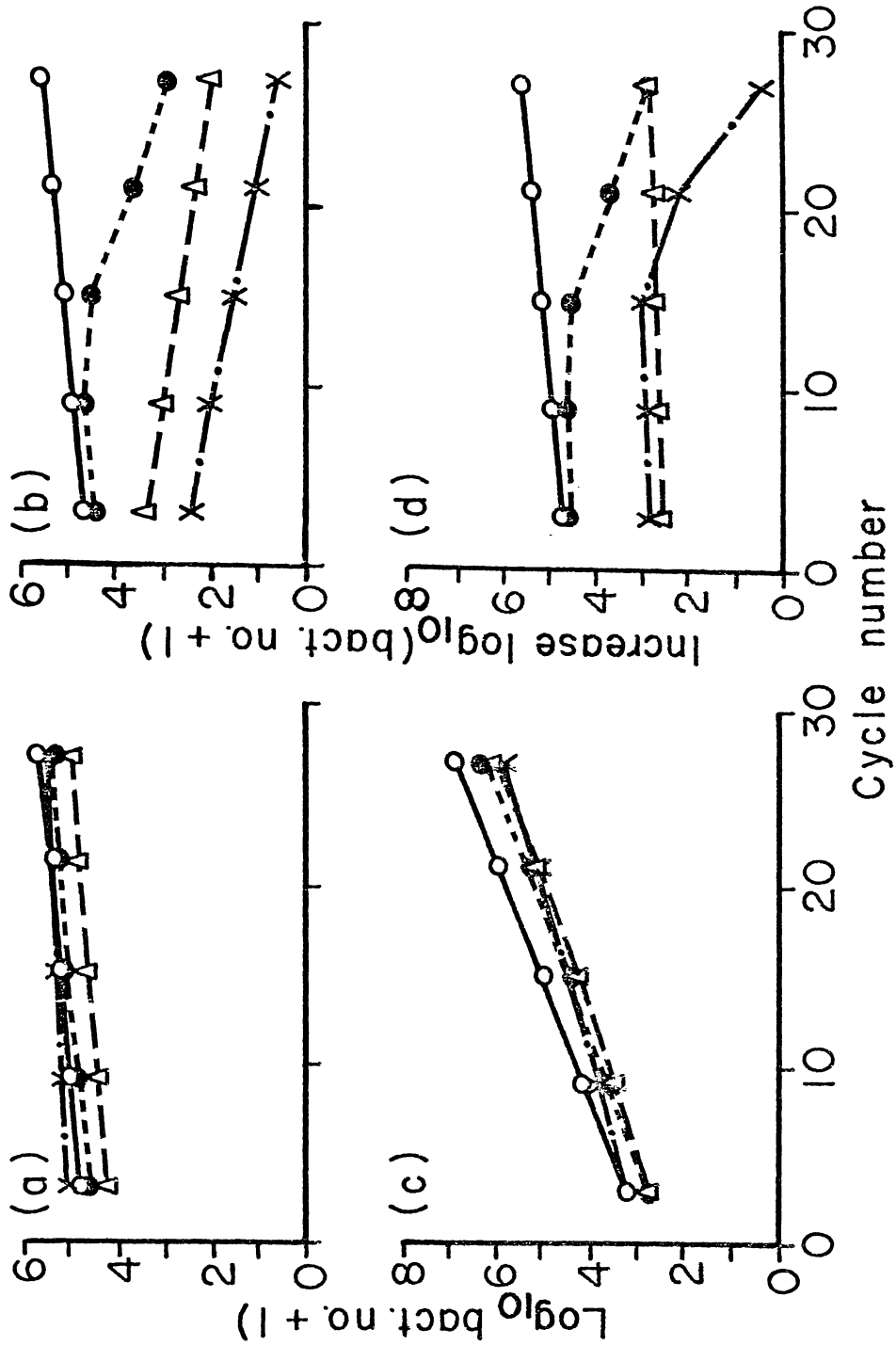

(a)
(b)
(c)
(d)
$\log_{10}$ bact. no. + 1)
Increase $\log_{10}$(bact. no. + 1)
Cycle number
0
2
4
6
8
10
20
30

Figure 2.3-7: Influence of cleaning system on effect of detergent and rinse on bacterial numbers on surfaces

(a) *S. faecalis* surviving detergent and rinse

(b) Decrease in *S. faecalis* with detergent and rinse

(c) *E. aerogenes* surviving detergent and rinse

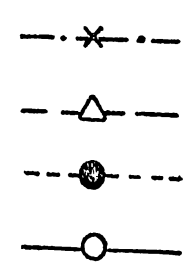
(d) Decrease in *E. aerogenes* with detergent and rinse

Key: System B.1 (no sanitizer)

System B.2 (pre-milk sanitizer)

System B.3 (post-wash sanitizer)

System B.4 (intercycle sanitizer)



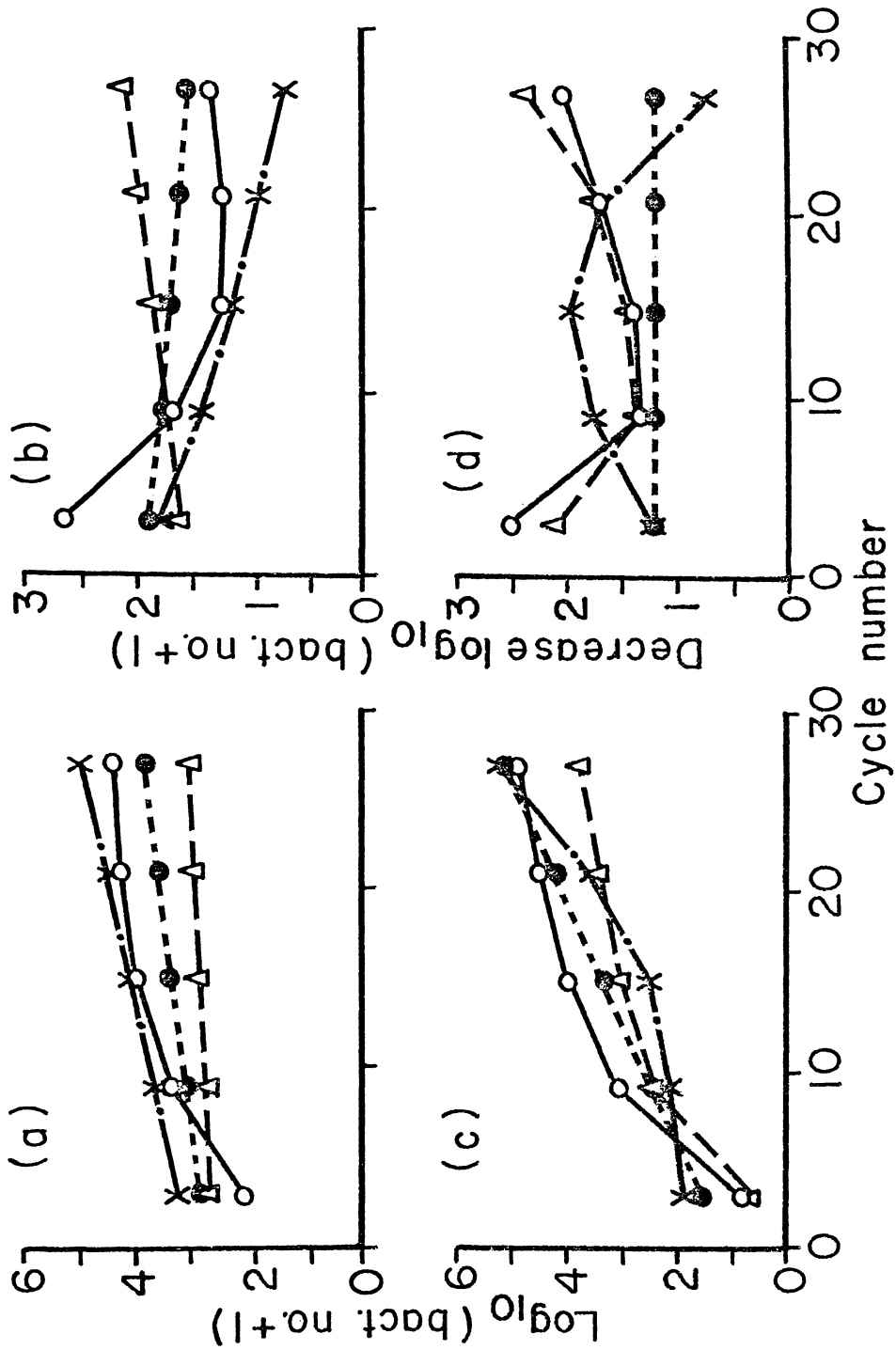

(a)
(b)
(c)
(d)
$\log_{10}$ (bact. no.+1)
Decrease $\log_{10}$ (bact. no.+1)
Cycle number
0
2
4
6
10
20
30
1
3

Figure 2.3-8: Influence of cleaning system on the effect of final solution on bacterial numbers on surfaces

(a) S. faecalis surviving final solution

(b) Decrease in S. faecalis with final solution

(c) E. aerogenes surviving final solution

(d) Decrease in E. aerogenes with final solution

Key: System B.1 (no sanitizer)

System B.2 (pre-milk sanitizer)

System B.3 (post-wash sanitizer)

System B.4 (intercycle sanitizer)

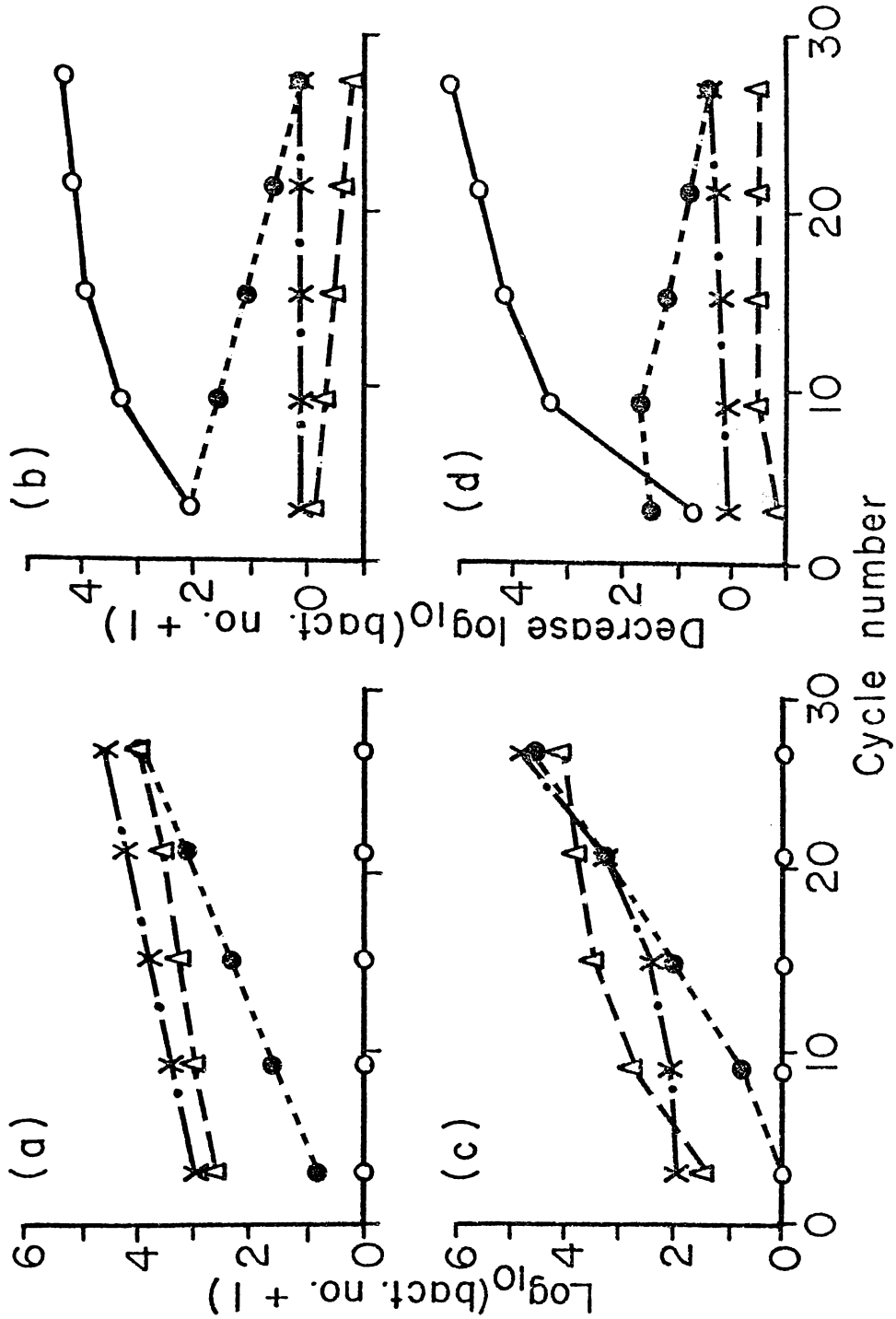

(a)
(b)
(c)
(d)
$\log_{10}$(bact. no. + 1)
Decrease $\log_{10}$(bact. no. + 1)
Cycle number
0
2
4
6
10
20
30

Figure 2.3-9: Influence of cleaning system on the effect of the intercycle period on bacterial numbers on surfaces

(a) S. faecalis surviving intercycle period

(b) Decrease in S. faecalis during intercycle period

(c) E. aerogenes surviving intercycle period

(d) Decrease in E. aerogenes during intercycle period

Key: System B.1 (no sanitizer)

System B.2 (pre-milk sanitizer)

System B.3 (post-wash sanitizer)

System B.4 (intercycle sanitizer)

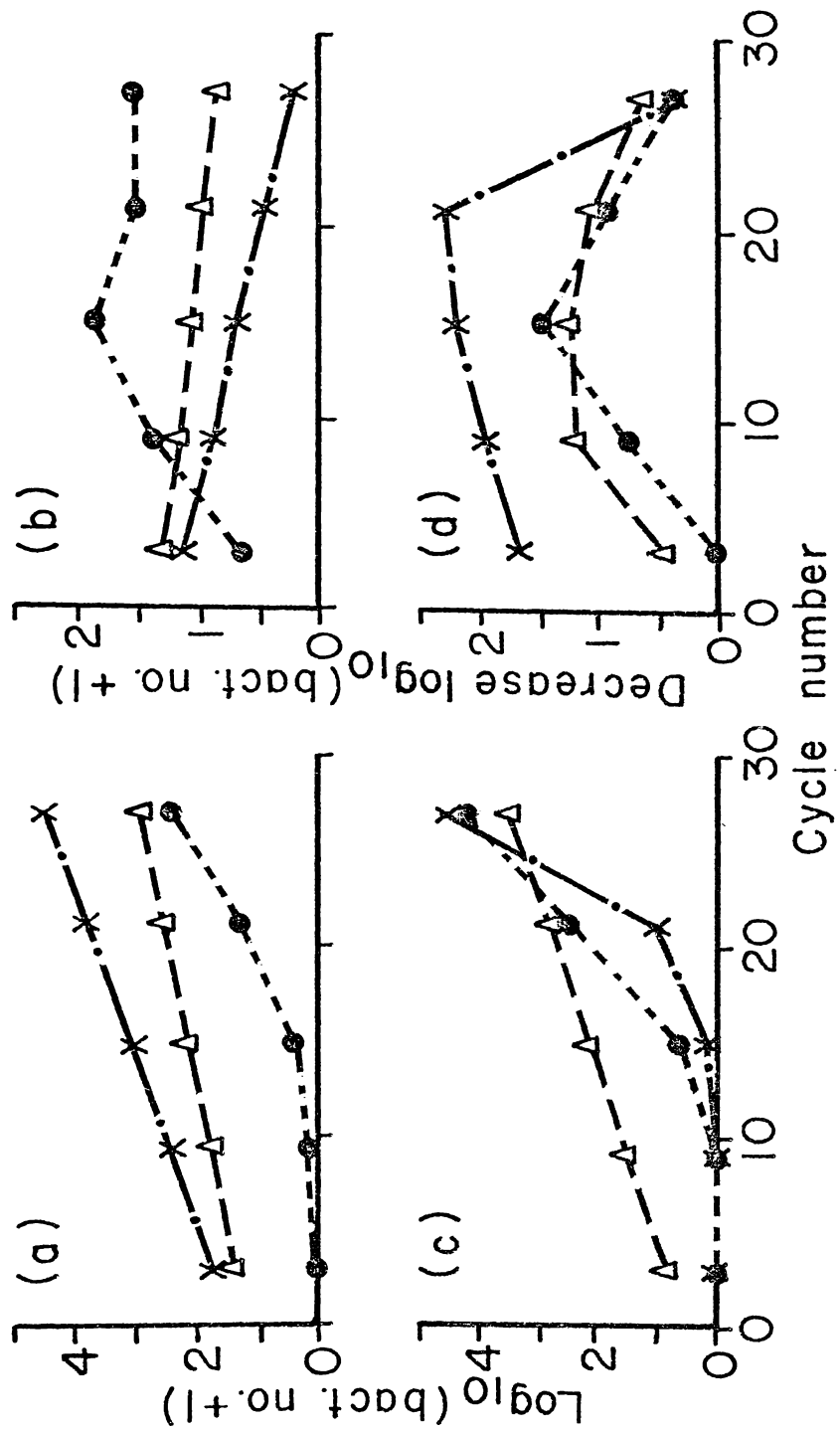

(a)
(b)
(c)
(d)
Log10 (bact. no.+1)
Decrease log10 (bact. no.+1)
Cycle number
0
2
4
10
20
30

Figure 2.3-10: Influence of cleaning system on the effect of the pre-milk solution on bacterial numbers on surfaces

(a) S. faecalis surviving pre-milk solution

(b) Decrease in S. faecalis with pre-milk solution

(c) E. aerogenes surviving pre-milk solution

(d) Decrease in E. aerogenes with pre-milk solution

Key: System B.1 (no sanitizer)

System B.2 (pre-milk sanitizer)

System B.3 (post-wash sanitizer)

System B.4 (intercycle sanitizer)

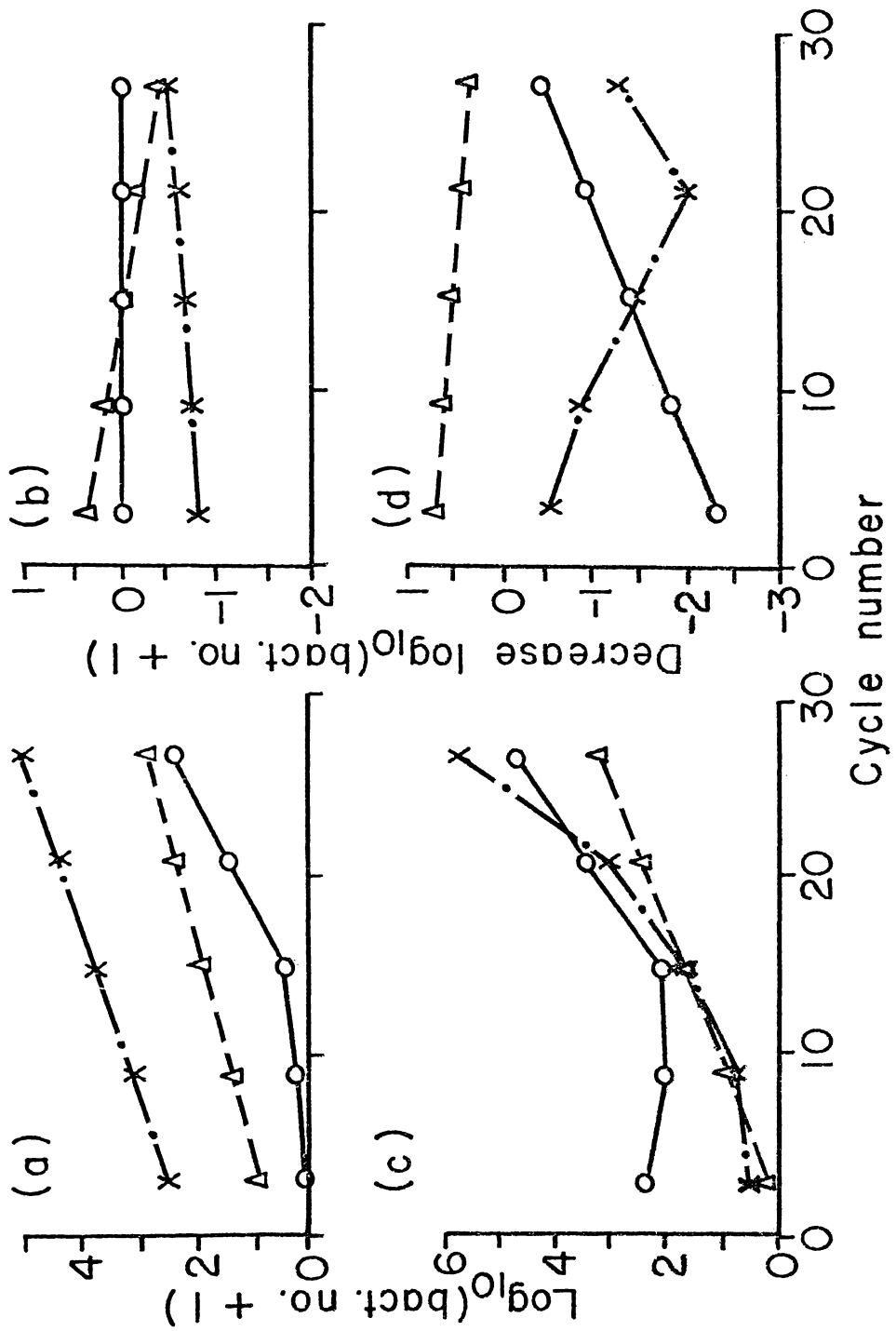

(a)
(b)
(c)
(d)
Log10(bact. no. + 1)
Decrease log10(bact. no. + 1)
Cycle number
0
2
4
6
10
20
30
-1
-2
-3

Figure 2.3-11: Influence of cleaning system on the effect of the sanitizer on bacterial numbers on surfaces

(a) *S. faecalis* surviving sanitizers

(b) Decrease in *S. faecalis* with sanitizers

(c) *E. aerogenes* surviving sanitizers

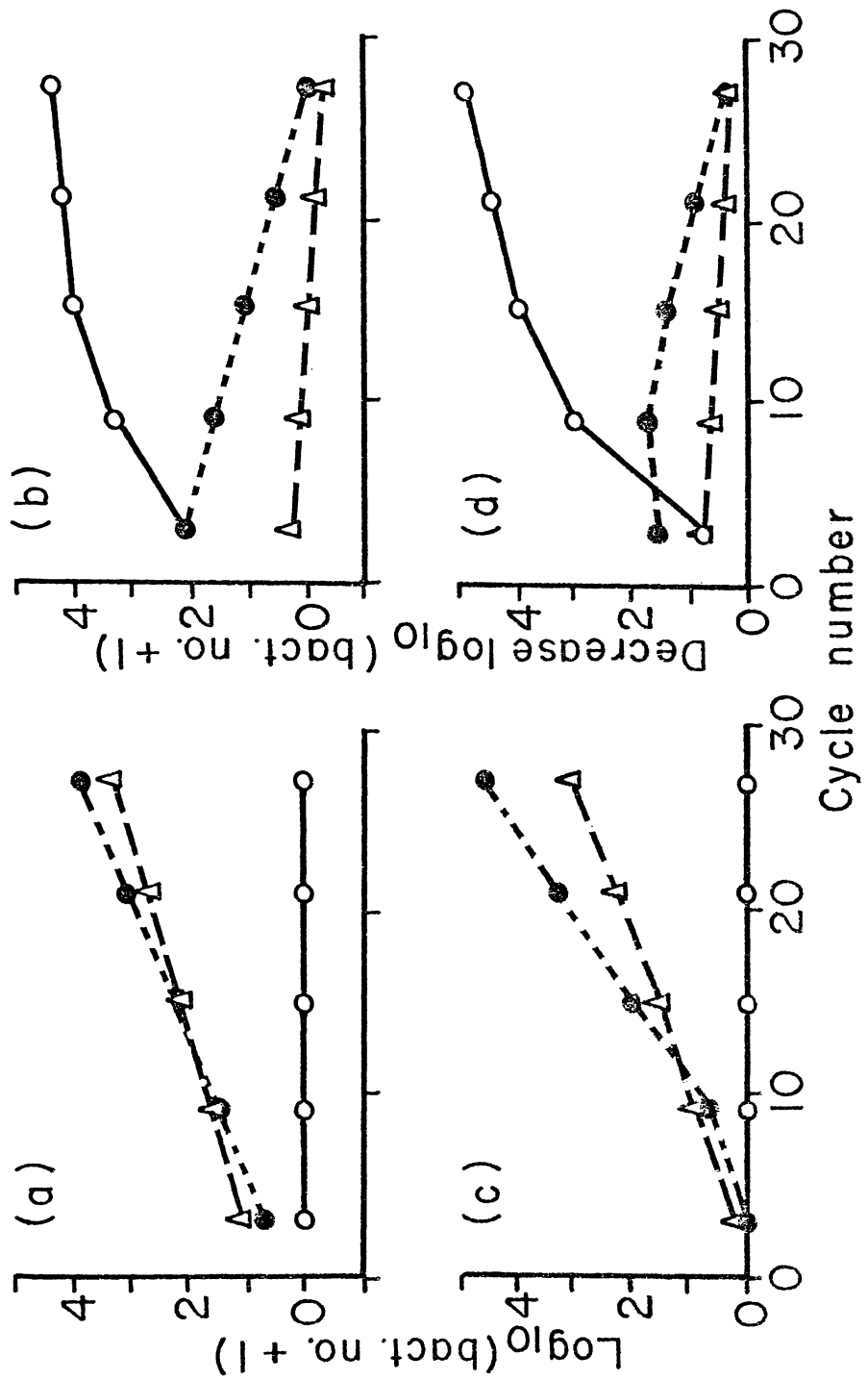
(d) Decrease in *E. aerogenes* with sanitizers

Key:

System B.2 (pre-milk sanitizer)

System B.3 (post-wash sanitizer)

System B.4 (intercycle sanitizer)


(a)
(b)
(c)
(d)
Log10 (bact. no. + 1)
Decrease log10 (bact. no. + 1)
0
2
4
0
10
20
30
Cycle number

B.2 (pre-milk sanitizer , Figures 2.3-3a and 2.3-3b). The final solution of water had a neglibible effect with the S. faecalis, but a contaminating effect with the E. aerogenes. There was a small decrease in numbers during the intercycle period at all cycle numbers. The iodophor sanitizer applied before milking had a small reducing effect on S. faecalis in early cycles, but no effect in later cycles. It had a reducing effect on E. aerogenes at all cycle numbers.

The detergent also greatly reduced the surface counts of S. faecalis (Figure 2.3-4a) and E. aerogenes (Figure 2.3-4b) in System B.3 (post-wash sanitizer). However, in this system the application of the iodophor in the post-wash step of the cycle enabled the iodophor sanitizer to reduce the count to an equal degree. In the intercycle period the numbers of both S. faecalis and E. aerogenes were reduced slightly at all cycle numbers. The pre-milk rinse of water had no effect on the count of S. faecalis but increased the count of E. aerogenes due to contaminated rinse water.

In System B.4 (intercycle sanitizer) the detergent rinse had a similar effect on the counts of S. faecalis (Figure 2.3-5a) and E. aerogenes (Figure 2.3-5b) as in the other systems. In this system, however, the application of the sanitizer for the whole of the intercycle period enabled it to be the most effective bacterial control agent in the system. The control of both S. faecalis and E. aerogenes was complete, there being no viable organisms of either species remaining at the time of the milk soiling (after drainage of the intercycle sanitizer) for the complete experimental period.

PERFORMANCE OF SYSTEM COMPONENTS

Milk Soiling

After milk soiling, the numbers of S. faecalis on the surfaces increased slowly with time, with minor differences between the treatments (Figure 2.3-6a). The numbers attaching at any time (Fig. 2.3-6b) were influenced by the amount of soil on the surface and the numbers on the surface after the pre-milk treatment. The numbers of E. aerogenes on the surface after milk soiling (Figure 2.3-6c) increased over time at a greater rate than those of S. faecalis. There was a greater count with System B.4, which had the greatest soil mass. The numbers attaching at any time during soiling were governed by the same factors as with S. faecalis, but the numbers changed due to contaminated pre-milking rinse water in Systems B.1 and B.3.

Effect of Detergent and Rinse

This treatment was common to all test cells, so any differences were due to the different components of these systems. The efficiency of the detergent, as shown by the numbers of survivors and the reduction in count, was inversely related to the amount of soil on the surface (Figures 2.3-7a to c). This was shown in both the relative performance of the detergent between systems and the reduction in detergent efficiency with increasing cycle number.

Effect of Final Solution

The final solutions in Systems B.1 and B.2 were both water rinses, and consequently had negligible effect upon the surface count of both organisms (Figure 2.3-8a to d). The application of a post-wash sanitizer in System B.3 significantly reduced the numbers

Figure 2.3-12: Influence of 4 cleaning systems on the numbers of bacteria on stainless steel surfaces

(a) S. faecalis surviving systems

(b) Decrease in S. faecalis with systems

(c) E. aerogenes surviving systems

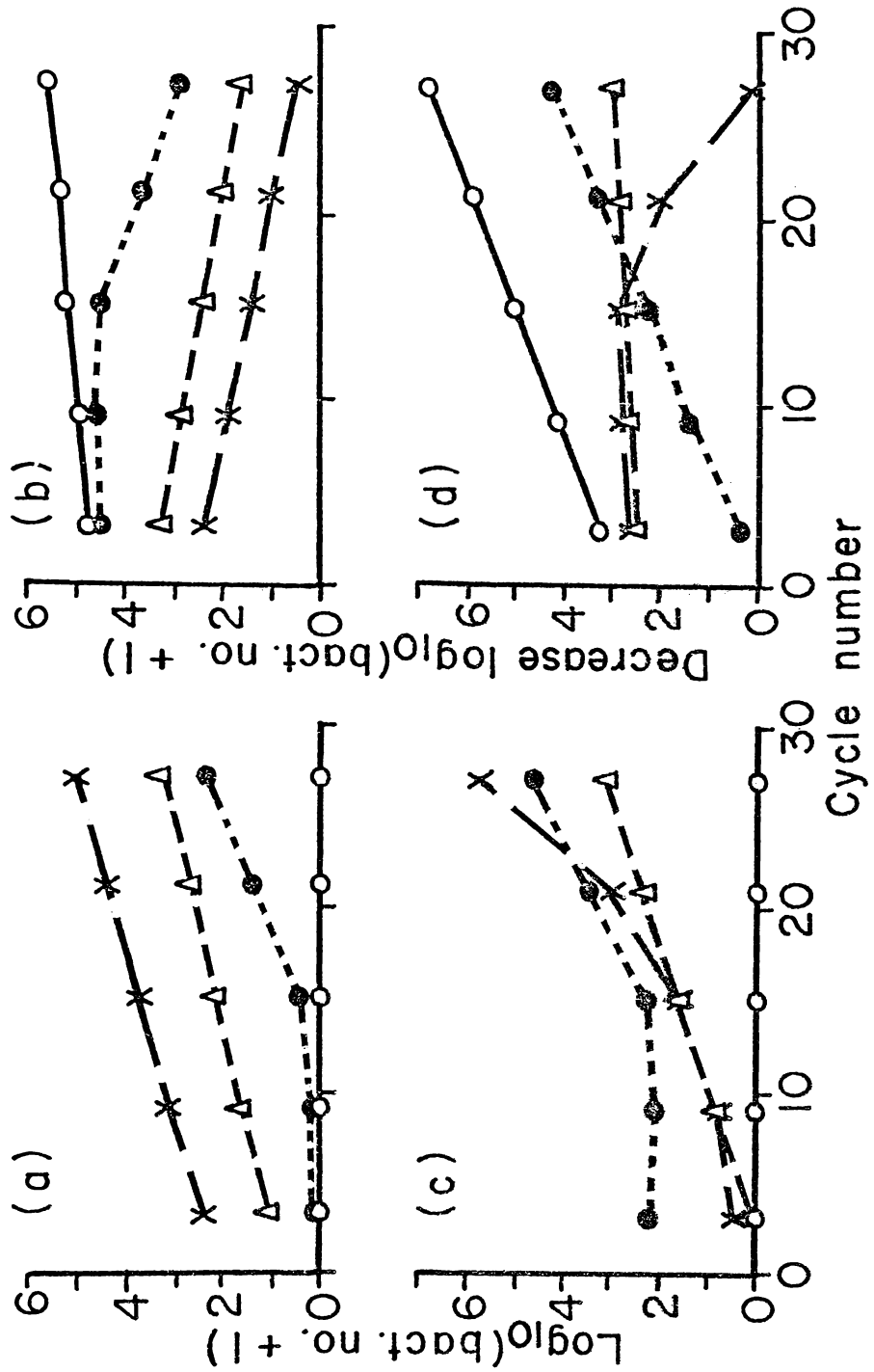
(d) Decrease in E. aerogenes with systems

Key: System B.1 (no sanitizer)

System B.2 (pre-milk sanitizer)

System B.3 (post wash sanitizer)

System B.4 (intercycle sanitizer)


(a)
(b)
(c)
(d)
Log10(bact. no. + 1)
Decrease log10(bact. no. + 1)
Cycle number
0
2
4
6
0
10
20
30

of both organisms in early cycles when the soil mass was low, but this effect diminished with time. The application of the intercycle sanitizer in System B.4 was totally effective in bacterial control at all cycle numbers.

Effect of Intercycle Period

The numbers of all organisms were reduced during the intercycle period (Figure 2.3-9a to d). This reduction was influenced by three factors; the amount of soil on the surface, the application of a sanitizer as the final treatment prior to the intercycle period (System B.3), and the organism - S. faecalis survived more effectively in early cycle numbers than did E. aerogenes. If the experiment had been extended in duration it is possible that growth may have occurred during the intercycle time with Systems B.1 and B.2. No data are reported for System B.4 as the sanitizer was in the test cell during the intercycle period.

Effect of Pre-Milk Solution

The pre-milk solution was a water rinse in systems B.1 and B.3, an iodophor rinse in system B.2 and the intercycle sanitizer in System B.4. The effect of the pre-milk solution is related to both the overall efficiency of the system (cf systems B.1 and B.3 in Figures 2.3-8a to d) and the use of a pre-milk sanitizer. The sanitizer efficiency was small in early cycles with S. faecalis, reducing to a level where the count was unchanged. The sanitizer had a greater effect on E. aerogenes (System B.2 in Figures 2.3-10b and 2.3-10d).

Effect of Sanitizer

The iodophor sanitizer was applied in three ways in the differ-

Figure 2.3-13: Influence of the components of System B.1 (no sanitizer) on the numbers of three groups of bacteria.

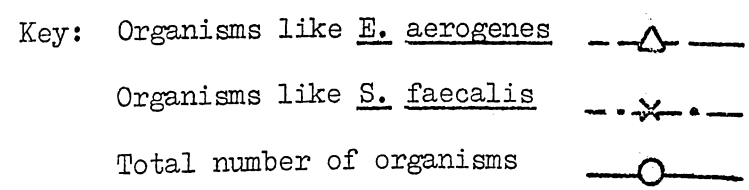
(a) After milk soiling

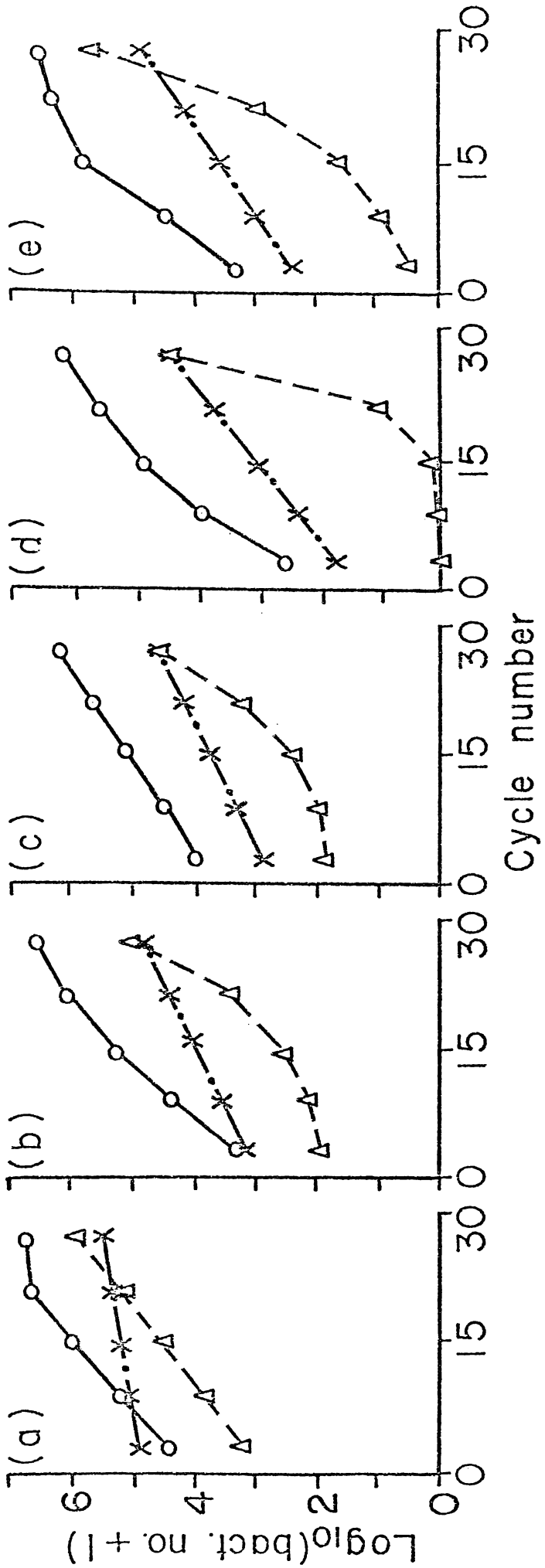
(b) After detergent and rinse

(c) After final solution

(d) After intercycle period

(e) After pre-milk solution




(a)
(b)
(c)
(d)
(e)
0
2
4
6
15
30
Log10(bact. no. + 1)
Cycle number

ent cleaning systems; as a pre-milking rinse in System B.2, as a post-wash rinse in System B.3 and as an intercycle solution in System B.4. Figures 2.3-11b and 2.3-11d show that those treatments were clearly different in their relative efficiencies.

The long exposure time of the intercycle sanitizer resulted in it being totally effective in bacterial control. The increase in the amount by which the bacterial numbers were reduced does not indicate that the efficiency of the sanitizer was increasing, but merely reflected the increasing numbers of organisms exposed to the sanitizer after the washing solutions had been drained (Figures 2.3-7a and 7c). It is probable that, if the experiment had been continued, the curves for both organisms would have reached a maximum and then decreased when the soil had accumulated to a depth where the sanitizer could not kill all of the organisms it contained.

Of the two systems where the iodophor was applied as a short contact-time rinse, the reductions in counts show the post-wash application (System B.3) was more effective than the pre-milk application (System B.2). The post-wash application may not give the bacteria an opportunity to recover from the rigours of the washing solutions, resulting in a more pronounced bactericidal effect. The pre-milk application gave the bacteria a 9.5 hr period to recover from the washing solution, which may have permitted them to survive the sanitizer more efficiently.

THE MICRO-ECOLOGY OF THE EQUIPMENT SURFACES

Both of the dose organisms colonised the surfaces of the substrate, but in different ways. S. faecalis attached in high

numbers in the early cycles and showed only a very small increase over time (Figure 2.3-3a). However, E. aerogenes attached in relatively small numbers in early cycles, but the attachment increased at a faster rate than with S. faecalis. This small attachment for an early period, and later high rate was also demonstrated when E. aerogenes was examined for its attachment to glass in a skim milk soil (Figure 2.1-6a).

The detergent effected similar reductions in numbers of both organisms, suggesting that the organisms were passively removed, rather than killed by this treatment (Figures 2.3-7b and d). It also appeared that the application of two different types of stress close together (alkaline detergent and iodophor sanitizer) was more effective than those same stresses applied with an intervening recovery period.

As in the experiment described in Section 2.2, there was no occasion during which the conditions were suitable for growth in the intercycle period. There were situations where there was no reduction in count, particularly where a large amount of soil had accumulated, but none where growth occurred. The major reason was that the relative humidity of 85% was still insufficient for the growth of the dose organisms used. The intercycle conditions were made even more unsuitable for growth by the presence of sanitizer residues on the surfaces (System B.3), and all organisms were killed by exposure to the sanitizer for the long intercycle period (System B.4). The organisms also differed in their ability to survive the intercycle period. E. aerogenes survived the intercycle period poorly in early cycles when the soil was low, but much more effectively in later cycle numbers (e.g. Figure 2.3-13d).

SUMMARY

The experiment has demonstrated that:

1. In terms of bacteriological performance, the sanitizer can be an important and, under some conditions, the most effective system component;
2. Of the short contact-time sanitizer applications, the post-wash sanitizer was more effective in reducing the bacterial count than the pre-milk sanitizer;
3. The application of the sanitizer for the complete intercycle period was totally effective in reducing the bacterial count and thus far more effective than either of the short contact-time methods;
4. The sanitizer can play an important role in the detergency of the system; and
5. Organisms varied according to species in their soiling characteristics and survival in intercycle conditions.

2.4 SIMULATOR INVESTIGATION OF COMPLEMENTARY CLEANING

As a milk soil contains both alkali-soluble (fat, protein) and acid-soluble (mineral) fractions it has been considered that the sequential use of alkaline and acidic detergents is essential for efficient cleaning. The acidic and alkaline mixtures are "complementary" detergents when used in this way. In most systems the alkaline detergent is used routinely, with acidic detergents applied from frequencies of the weekly (Whittlestone, 1952) to "whenever necessary".

Scott, et al. (1962) incorporated an iodophor sanitizer into a system as a pre-milking operation at every milking. The acidic-detergent nature of the iodophor was used to eliminate the need for the weekly acidic treatment.

The following experiment was performed to investigate:

1. Whether complementary cleaning is advantageous in a soak-cleaning situation;
2. The nature of the detergency and sanitizing performance of an iodophor; and
3. The difference between rubber and stainless steel as substrates.

2.4.1 EXPERIMENTAL DESIGN

The four systems examined are presented in table 2.4-1. This table shows that in System C.2 (no acid) the milk soiling was followed by a wash with a chlorinated-alkaline detergent, then a rinse of cold water, which was followed by a final solution of cold water. The intercycle period of 9.5h then commenced, and when completed was followed by milk soiling - there was no pre-milk rinse.

Table 2.4-1: Components of the 4 cleaning systems and sampling times in complementary cleaning study.

System		System components				
Number	Name	Milk	Detergent	Rinse	Final solution	Inter-cycle
C.1	Periodical acid	+	Chlorinated-alkaline 5 g/l, 20°C	Water 20°C	Water for 8 cycles, 20°C Acidic detergent every 9th cycle, 2 ml/l, 20°C	- -
C.2	No acid	+	Chlorinated-alkaline 5 g/l, 20°C	Water 20°C	Water 20°C	-
C.3	All acid	+	Chlorinated-alkaline 5 g/l, 20°C	Water 20°C	Acidic detergent, 2 ml/l, 20°C	-
C.4	Iodophor	+	Chlorinated-alkaline 5 g/l, 20°C	Water 20°C	Iodophor, 2 ml/l, 20°C	-
Sampling points *		I, G	M	R	F	

* I bacteriological count after intercycle period (before milk soiling)

M bacteriological count after milk soiling (before detergent),

R bacteriological count after detergent and rinse (before final solution),

F bacteriological count after final solution (before intercycle period),

G gravimetric assessment of soil after intercycle period (before milk soiling).

System C.1 (periodical application of acid detergent) was the same as C.2, except that approximately every 9th cycle an acidic detergent was substituted for the water as the final solution. In System C.3 (all acid) the same acidic detergent was used as the final solution, but in every cycle. In System C.4 (iodophor) the final solution used was an iodophor at every cycle. The chlorinated-alkaline detergent used was "AL-1" supplied by Alfa-Laval, NZ and applied at a use concentration of 5 g/l (200 mg available Cl/l). The iodophor used was "2-4-6" (2% I, 46% H_3PO_4) supplied by Wellcome NZ Ltd, and applied at 2 ml/l (20 mg Av.I/l). The acidic detergent was also supplied by Wellcome NZ Ltd and had an identical formulation to the iodophor, except that it contained no iodine. It was also applied at 2 ml/l.

The experimental treatments were applied to steel and rubber slides for 28 cycles, with samplings performed at cycles 2, 10, 11, 16, 17, 20, 27 and 28 for System C.1 and at cycles 2, 10, 15, 20 and 25 for the other systems. At each sampling quadruplicate steel slides were removed for surface bacterial counts and gravimetric assessment of soil at the times shown in Table 2.4-1. At each sampling cycle quadruplicate samples of rubber slides were taken for both surface bacterial counts and soil mass at the intercycle sampling point only with Systems C.2, C.3 and C.4. Microflora samples were taken at the intercycle sampling for both the steel and rubber slides.

The test cells were maintained at a temperature of 30°C and relative humidity of 90%. The dosing organisms used were *S. faecalis* and *E. coli*, each at a rate of 5×10^5/ml in the soiling milk. The only contaminating organisms in the cleaning solutions were some in

Figure 2.4-1a: Changes in mass of soil on stainless steel surfaces washed by four cleaning systems in the complementary cleaning study

Key: System C.1 (periodical acid)
System C.2 (no acid)
System C.3 (all acid)
System C.4 (iodophor)

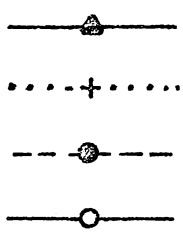
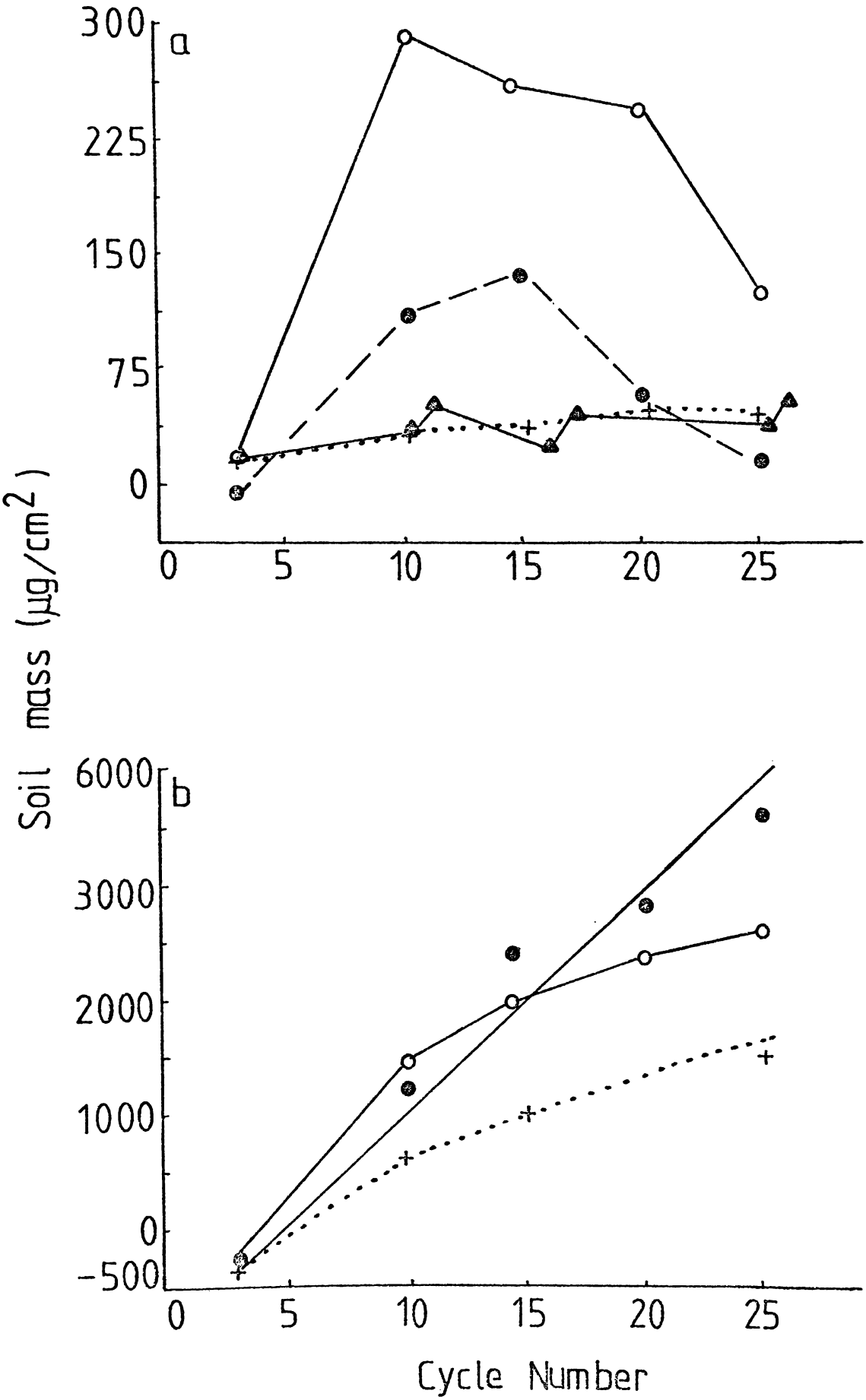


Figure 2.4-1b: Changes in mass of soil on rubber surfaces washed by four cleaning systems in the complementary cleaning study

Key: as for 2.4-1a


a
300
225
150
75
0
0
5
10
15
20
25
b
6000
3000
2000
1000
0
-500
0
5
10
15
20
25
Soil mass (µg/cm²)
Cycle Number

the rinse waters which did not grow on the selective media used for S. faecalis or E. coli.

2.4.2 RESULTS

The changes in soil mass on the steel and rubber surfaces with the four treatments are presented in Figures 2.4-1a and 1b. The data for both surfaces show that wherever the acid was used at every cycle in the cleaning system (Systems C.3 and C.4), the soil accumulation was greater than that when no acid was applied (System C.2). When the acid was applied to steel only periodically, there was a small immediate increase in the deposit present, although over the whole duration of the experiment there was no difference between the periodic-acid and no-acid systems (C.1 and C.2). The decrease in soil at higher cycle numbers with Systems C.3 and C.4 on steel suggested that the soil was accumulating to such a level that "peeling" from the surfaces was occurring.

The changes over 28 cycles in the numbers of viable S. faecalis on steel surfaces at the various stages of the cleaning system are presented in three-dimensional format in Figures 2.4-2a,-3a,-4a and -5a. The equivalent data for E. coli are presented in Figures 2.4-2b,-3b,-4b and-5b. The changes in numbers of these organisms at the end of the intercycle time on rubber and steel surfaces are presented in Figures 2.4-6a,-6b and-6c.

The influence of cleaning system components on the numbers of S. faecalis and E. coli on the steel surfaces are presented in Figures 2.4-7 (milk soiling), 2.4-8 (detergent and rinse), 2.4-9 (final solution) and 2.4-10 (intercycle time). The effects of complementary acid treatments on total system performance are

Figure 2.4-2a: Changes in numbers of S. faecalis on stainless steel surfaces over 28 cycles of System C.1 (periodical acid)

Figure 2.4-2b: Changes in numbers of E. coli on stainless steel surfaces over 28 cycles of System C.1 (periodical acid)

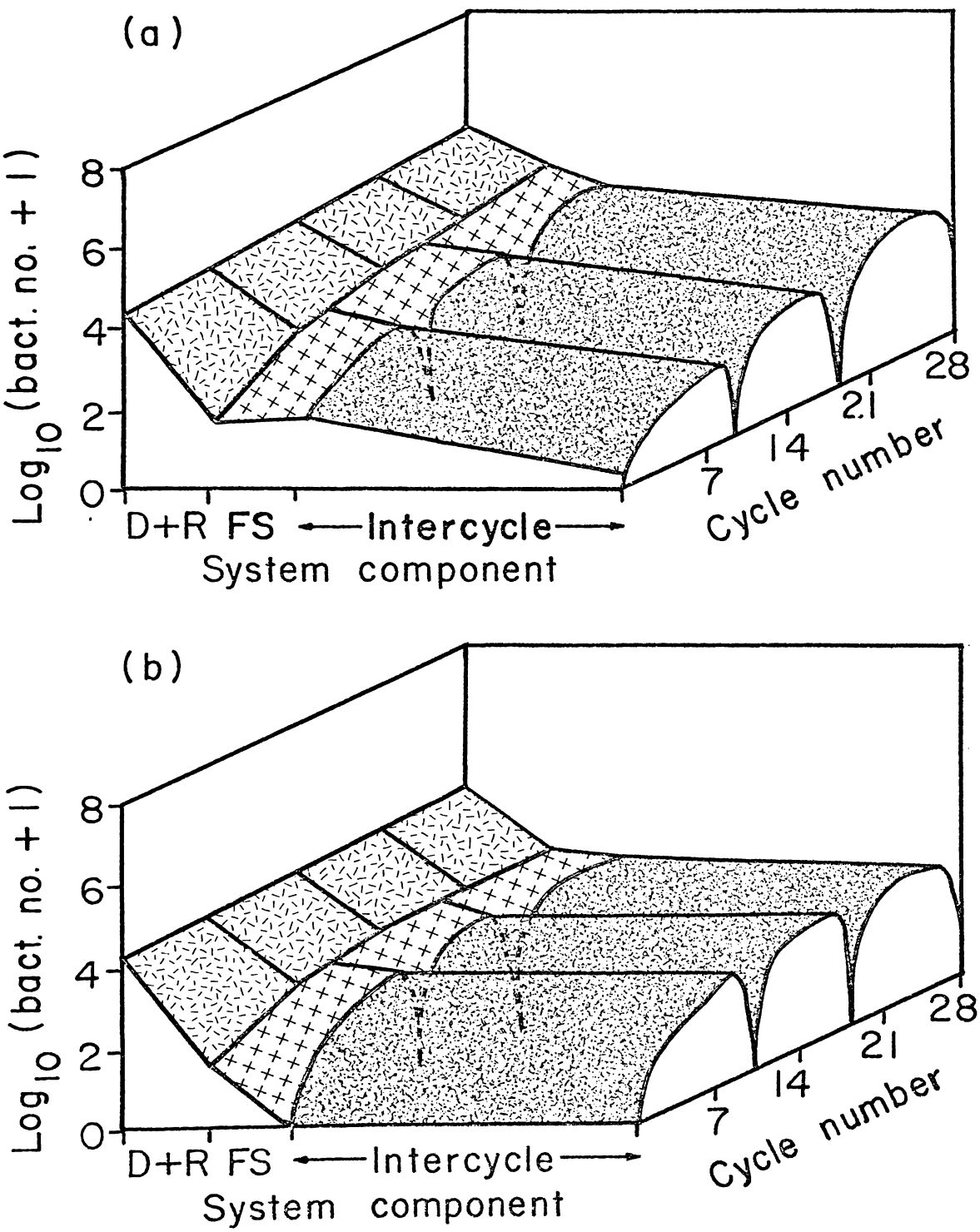
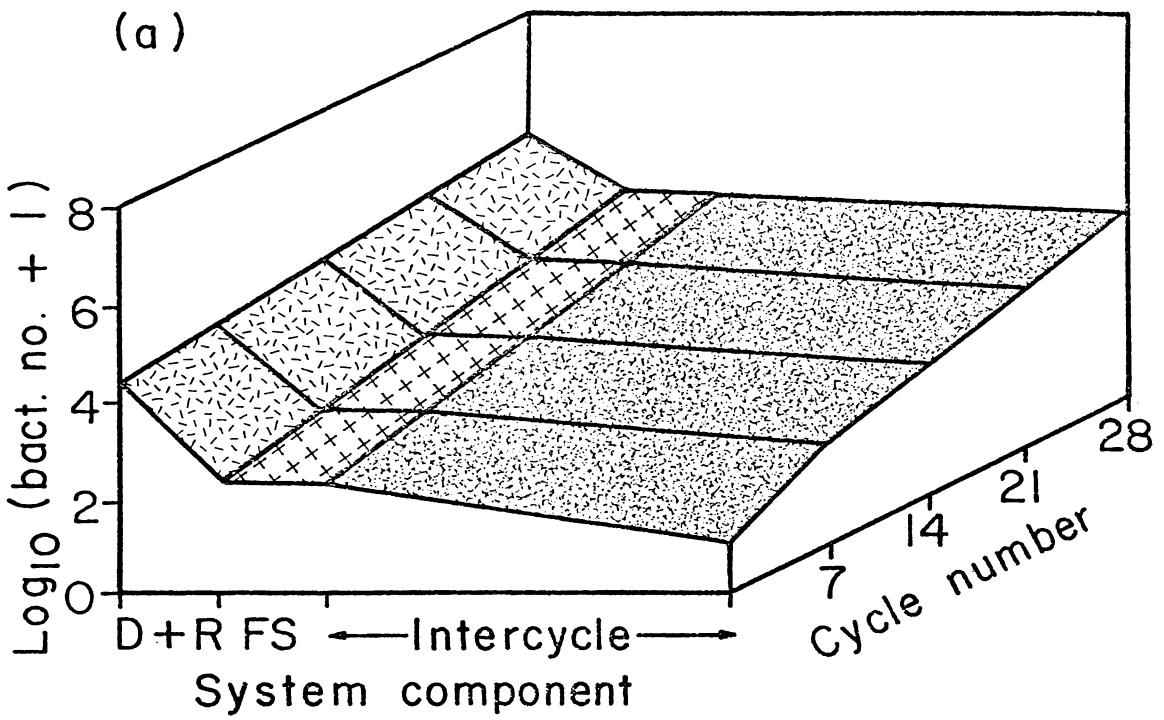

(a)
Log_{10} (bact. no. + 1)
8
6
4
2
0
D+R FS
Intercycle
System component
7
14
21
28
Cycle number
(b)
Log_{10} (bact. no. + 1)
8
6
4
2
0
D+R FS
Intercycle
System component
7
14
21
28
Cycle number

Figure 2.4-3a: Changes in numbers of S. faecalis on stainless steel surfaces over 28 cycles of System C.2 (no acid).

Figure 2.4-3b: Changes in numbers of E. coli on stainless steel surfaces over 28 cycles of System C.2 (no acid)


(a)
Log10 (bact. no. + 1)
0
2
4
6
8
D+R FS
Intercycle
System component
7
14
21
28
Cycle number

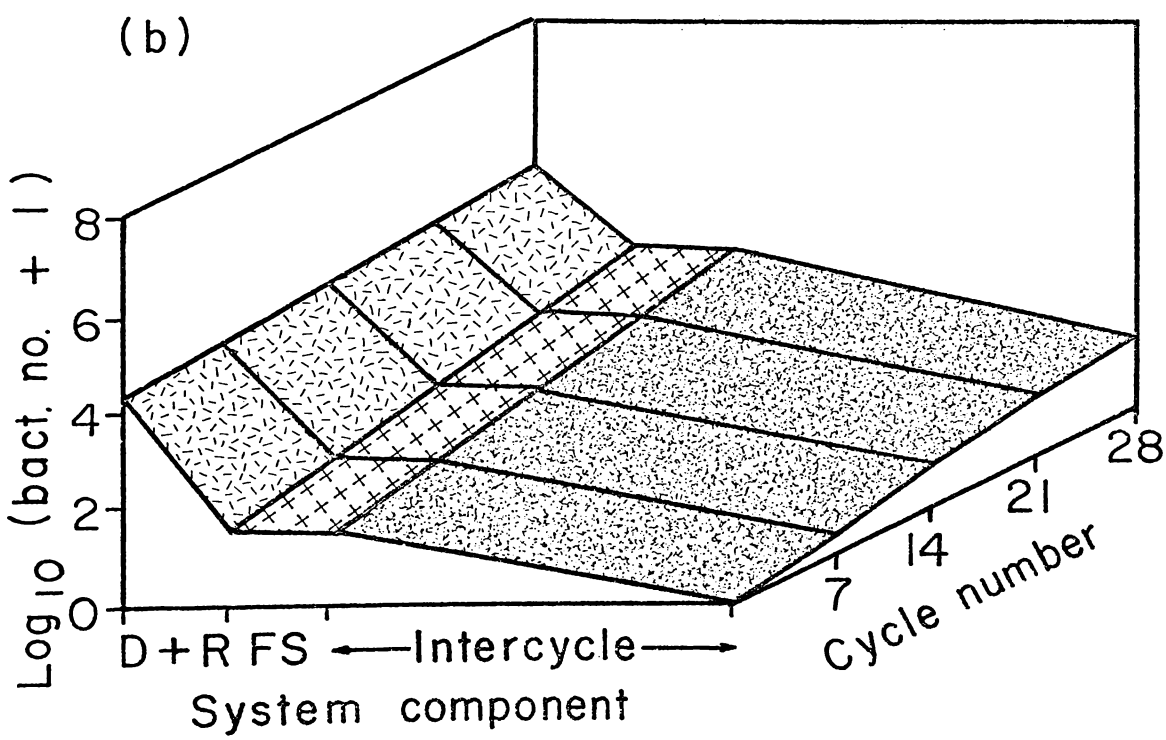
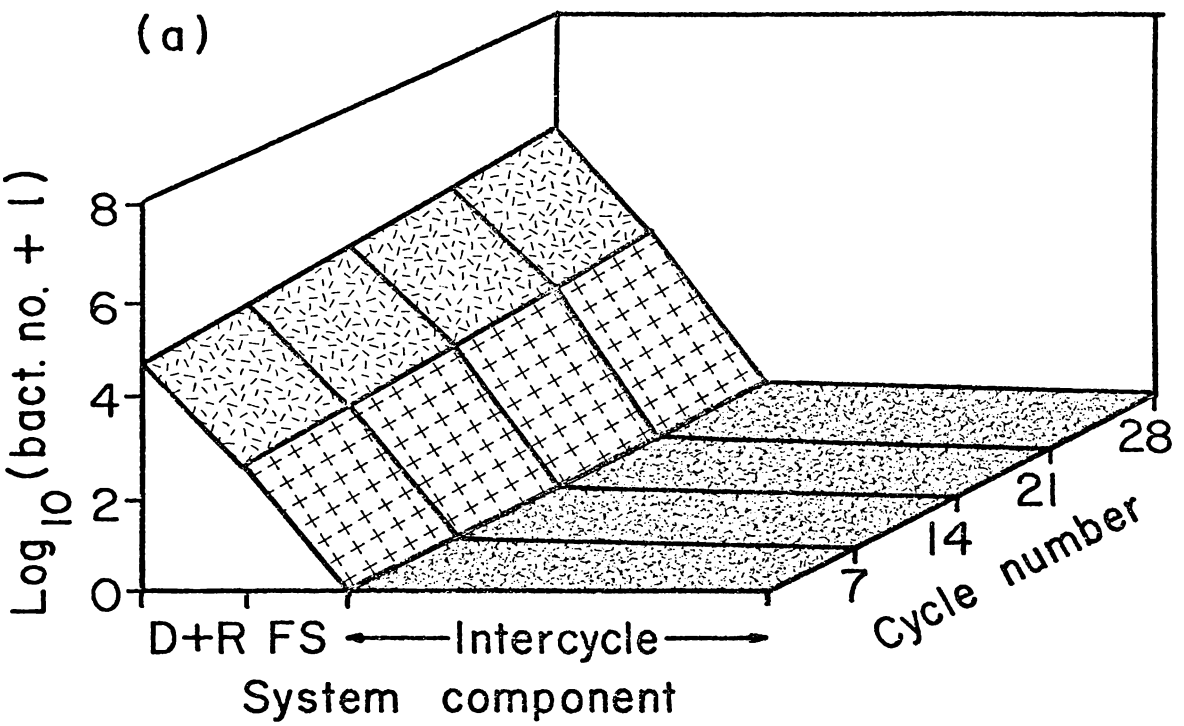

(b)
Log10 (bact. no. + 1)
0
2
4
6
8
D+R FS
Intercycle
System component
7
14
21
28
Cycle number

Figure 2.4-4a: Changes in numbers of S. faecalis on stainless steel surfaces over 28 cycles of System C.3 (all acid)

Figure 2.4-4b: Changes in numbers of E. coli on stainless steel surfaces over 28 cycles of System C.3 (all acid)


(a)
Log10 (bact. no. + 1)
8
6
4
2
0
D+R FS
Intercycle
System component
7
14
21
28
Cycle number

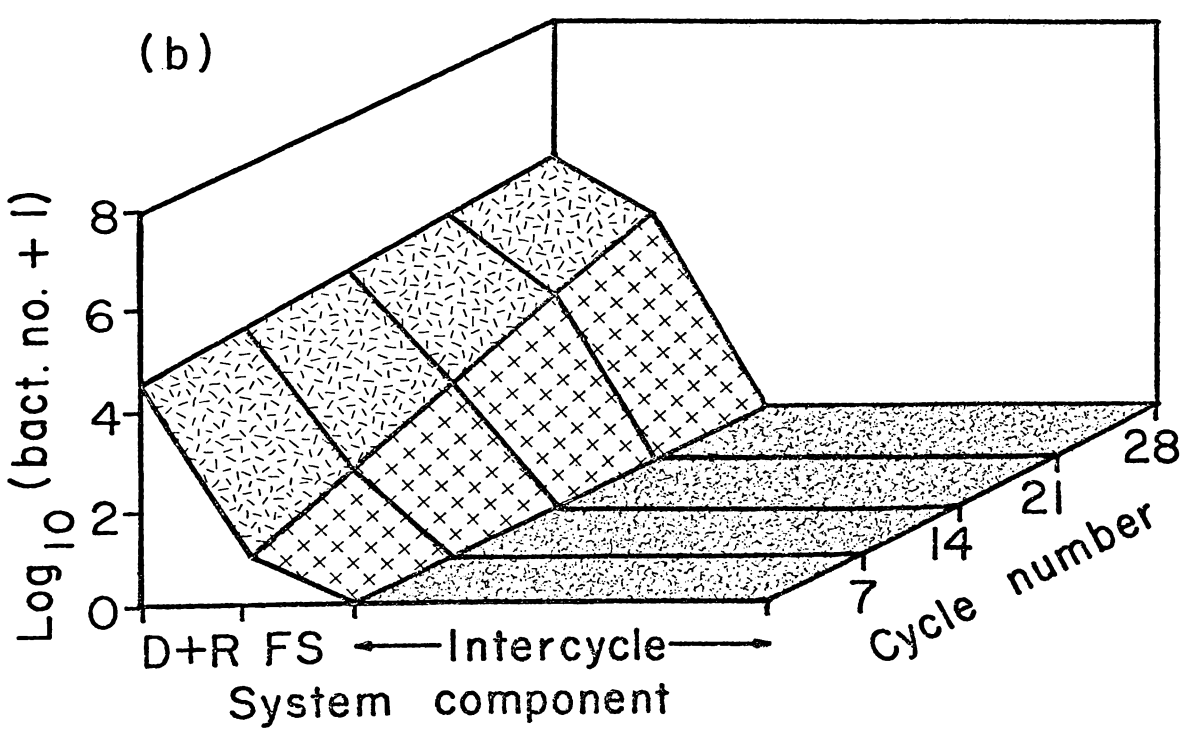
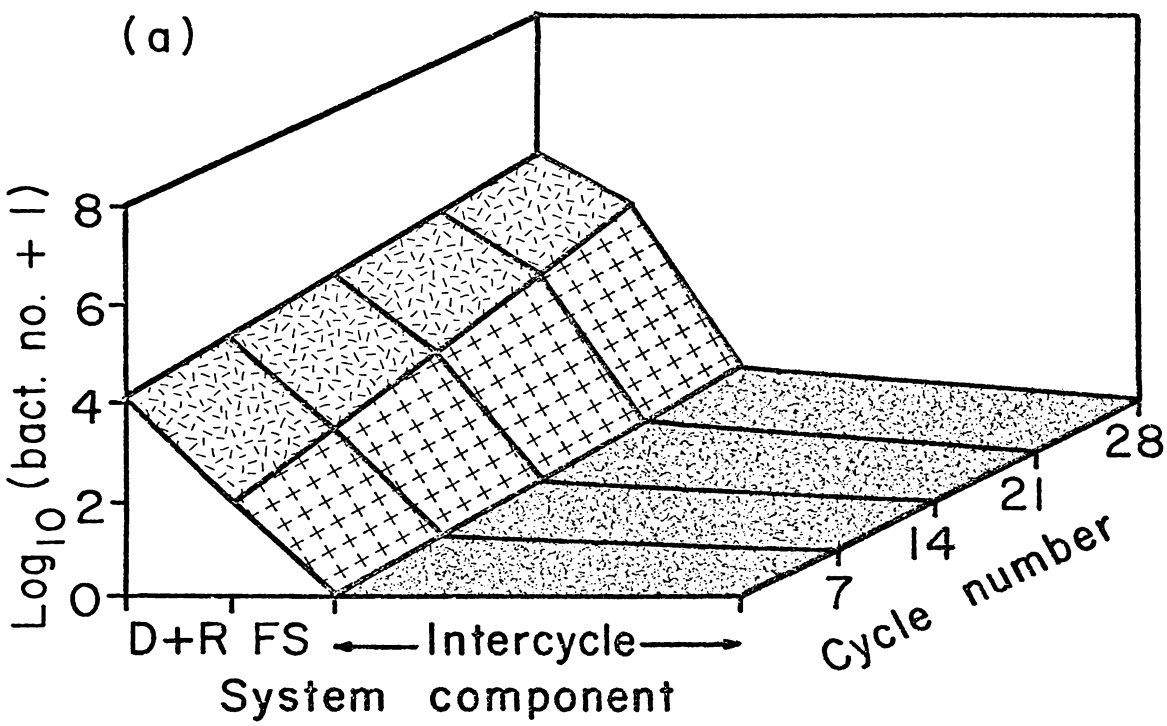
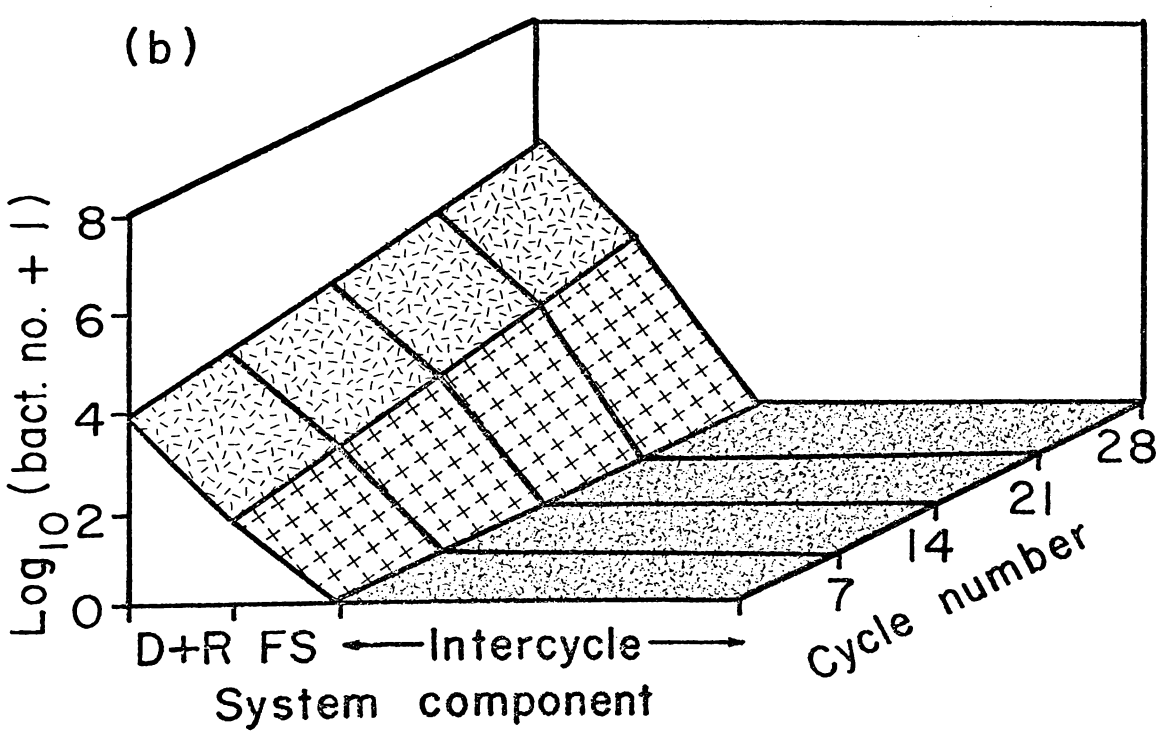

(b)
Log10 (bact. no. + 1)
8
6
4
2
0
D+R FS
Intercycle
System component
7
14
21
28
Cycle number

Figure 2.4-5a: Changes in numbers of *S. faecalis* on stainless steel surfaces over 28 cycles of System C.4 (iodophor)

Figure 2.4-5b: Changes in numbers of *E.coli* on stainless steel surfaces over 28 cycles of System C.4 (iodophor)


(a)
Log_{10} (bact. no. + 1)
0
2
4
6
8
D+R FS
Intercycle
System component
7
14
21
28
Cycle number


(b)
Log_{10} (bact. no. + 1)
0
2
4
6
8
D+R FS
Intercycle
System component
7
14
21
28
Cycle number

presented in Figure 2.4-11. In all of these figures both the numbers of survivors and the change in count due to the component or system are presented for both organisms.

The total numbers of organisms, and the numbers of S. faecalis and E. coli at each stage of System C.2 (no acid) are presented in Figure 2.4-12. As the information obtained for the other systems was very similar to that of C.2, only that for C.2 is presented.

The relative numbers of groups of organisms in the microflora of steel and rubber surfaces at times through the experimental period are presented in Table 2.4-2. The low numbers of organisms on the steel surfaces after the intercycle period with Systems C.3 and C.4 prevented the determination of the surface microflora.

2.4.3 DISCUSSION

PERFORMANCE OF THE TOTAL SYSTEMS

On both steel and rubber surfaces the addition of a complementary acidic detergent to the system (Systems C.3 and C.4) increased the soil accumulation compared to the no-acid system (System C.2, Figure 2.4-1). However, there may have been an interaction between the substrate and system, as on steel surfaces the acidic detergent gave greater deposit than the iodophor, while on rubber this order was reversed.

On steel surfaces in System C.2 (no acid) the detergent was the major component reducing the count of S. faecalis, the final rinse of water having no effect, and the intercycle period causing a small decrease (Figure 2.4-3a). This system affected E. coli similarly (Figure 2.4-3b), except that the reduction in the intercycle period was more pronounced.

Figure 2.4-6a: Changes over time in the numbers of S. faecalis and E. coli on rubber and stainless steel surfaces with System C.2 (no acid)

Key: S. faecalis on steel

S. faecalis on rubber

E. coli on steel

E. coli on rubber

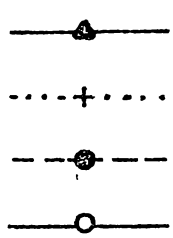
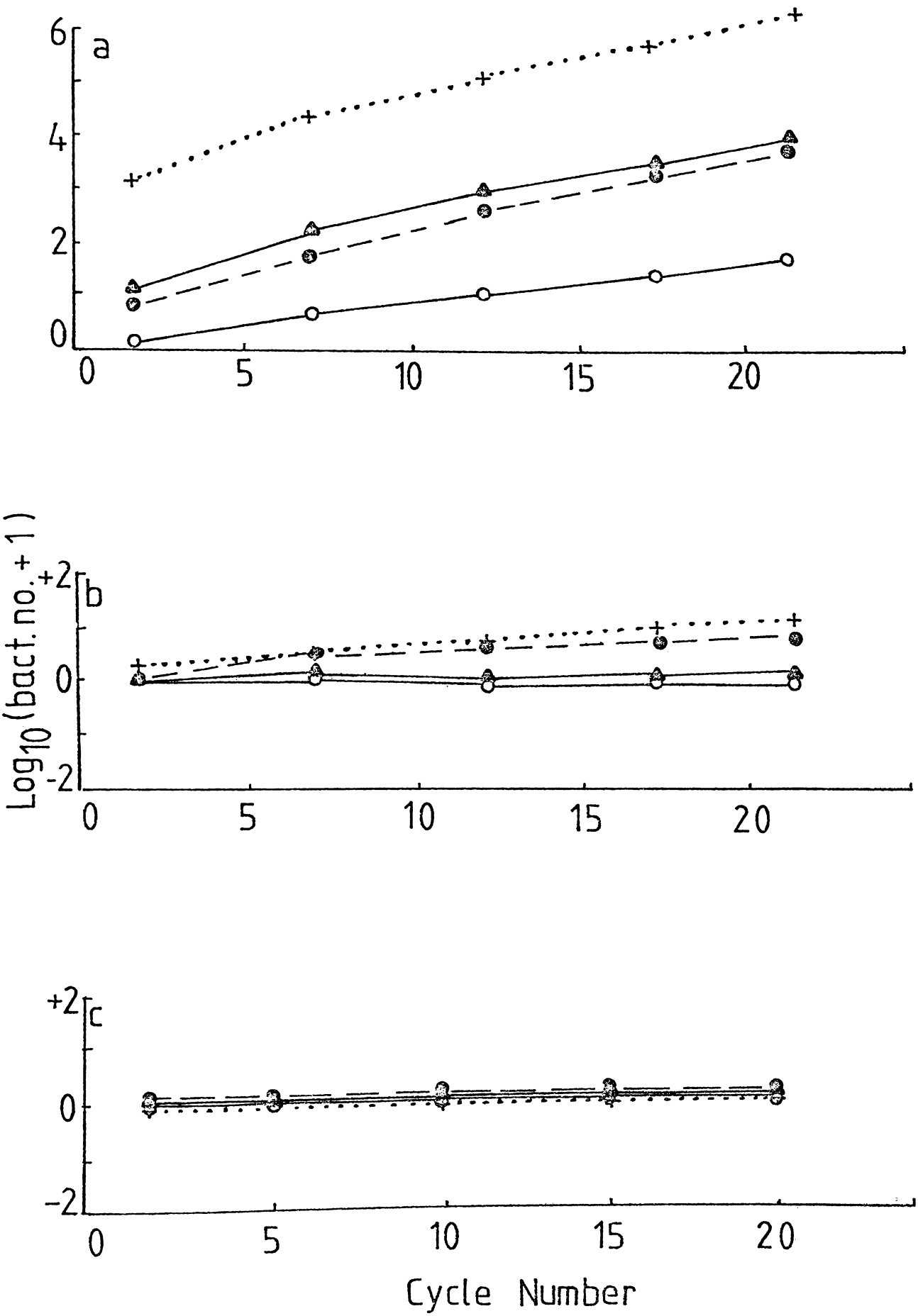


Figure 2.4-6b: Changes over time in the numbers of S. faecalis and E. coli on rubber and stainless steel surfaces with System C.3 (all acid)

Key: as for 2.4-6a

Figure 2.4-6c: Changes over time in the numbers of S. faecalis and E. coli on rubber and stainless steel surfaces with System C.4 (iodophor)

Key: as for 2.4-6a


a
b
c
6
4
2
0
+2
0
-2
0
5
10
15
20
Log10 (bact. no. + 1)
Cycle Number

Although the periodic application of an acidic detergent to steel slides in system C.1 (Figures 2.4-2a, 2b) had little effect on the soil mass, it had a marked influence on the surface bacterial numbers. The numbers of both S. faecalis and E. coli were reduced to zero by the acidic detergent, but only for the cycle during which it was applied. The numbers quickly returned to the level they had reached before the acid rinse. There was a small reduction in count during the intercycle period, but the numbers at the end of this period primarily reflected the count after the final solution.

Systems C.3 (all acid) and C.4 (iodophor) affected the surface S. faecalis counts very similarly (Figures 2.4-4a,5a). The detergent again reduced counts significantly. However, the final solution of acid or iodophor reduced the counts by a larger amount - to zero in early cycles and to low levels in later cycles. In the instances in which any organisms survived the final solution, they died in the subsequent intercycle period, leaving no viable organisms on the surfaces at the end of that period when the next milk soiling occurred. These two systems affected E. coli similarly (Figures 2.4-4b,-5b), except that the final solutions of acid and iodophor were able to kill or remove all of these organisms, as none were detected at the start of the intercycle period.

The data for steel surfaces in Figure 2.4-11a show that with S. faecalis Systems C.3 and C4 (acid and iodophor systems) were more efficient than Systems C.1 and C.2 (no-acid and periodical acid systems). With E. coli (Figure 2.4-11c) the order was slightly different, with the System C.2 (nc acid) having lower counts than System C.1 (periodical acid). Despite these minor variations, the bacterial performance reflected the bactericidal effect of the

Figure 2.4-7: Influence of cleaning system on effect of milk soiling on bacterial numbers on stainless steel surfaces

(a) S. faecalis surviving milk soiling

(b) Increase in S. faecalis with milk soiling

(c) E. coli surviving milk soiling

(d) Increase in E. coli with milk soiling

Key: System C.1 (periodical acid)

System C.2 (no acid)

System C.3 (all acid)

System C.4 (iodophor)

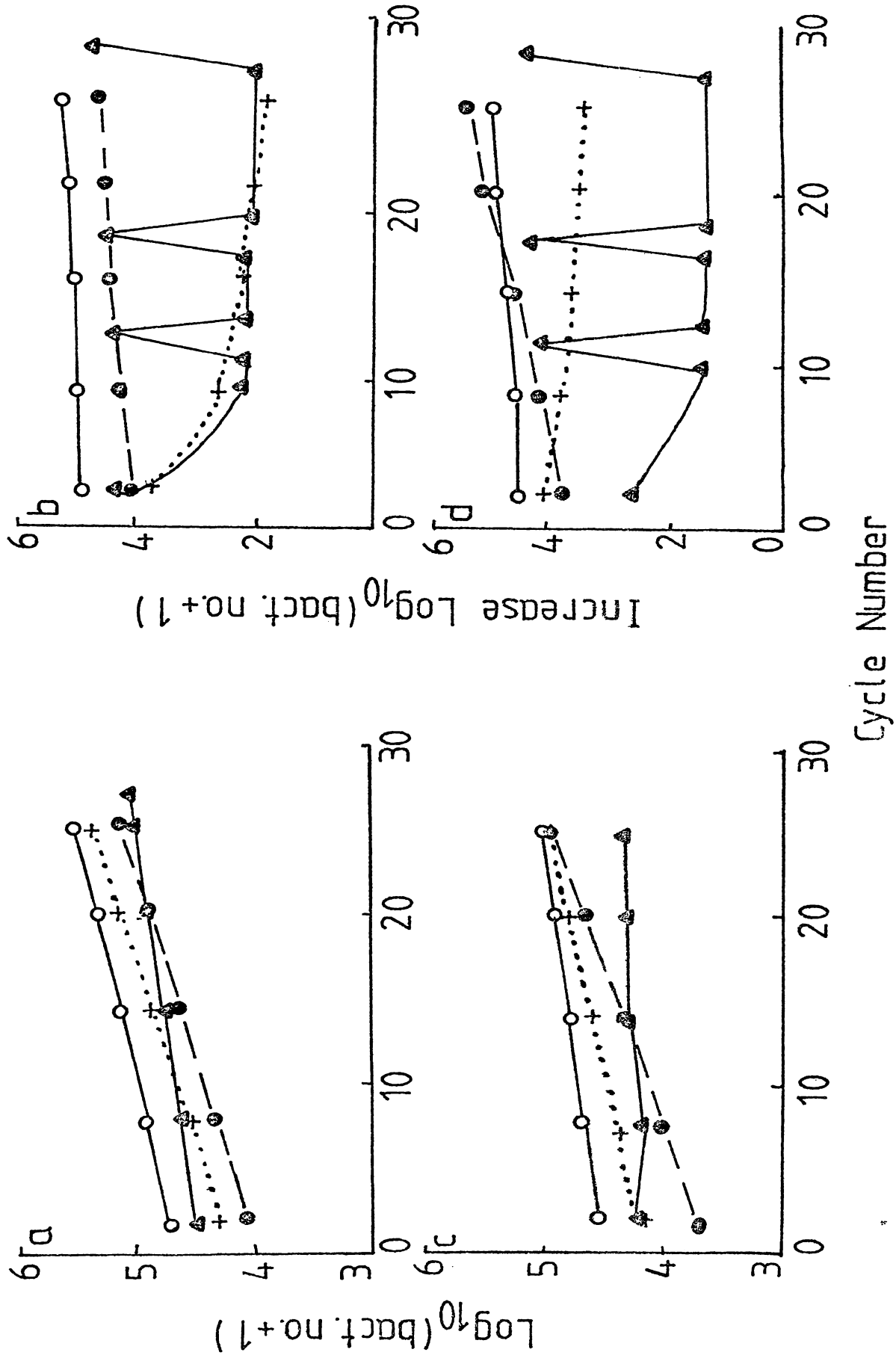

a
b
c
d
Log10(bact. no.+1)
Increase Log10(bact. no.+1)
Cycle Number
0
10
20
30
2
4
3
5
6

Figure 2.4-8: Influence of cleaning system on the effect of detergent and rinse on bacterial numbers on stainless steel surfaces

(a) S. faecalis surviving detergent and rinse

(b) Decrease in S. faecalis with detergent and rinse

(c) E. coli surviving detergent and rinse

(d) Decrease in E. coli with detergent and rinse

Key: System C.1 (periodical acid)

System C.2 (no acid)

System C.3 (all acid)

System C.4 (iodophor)

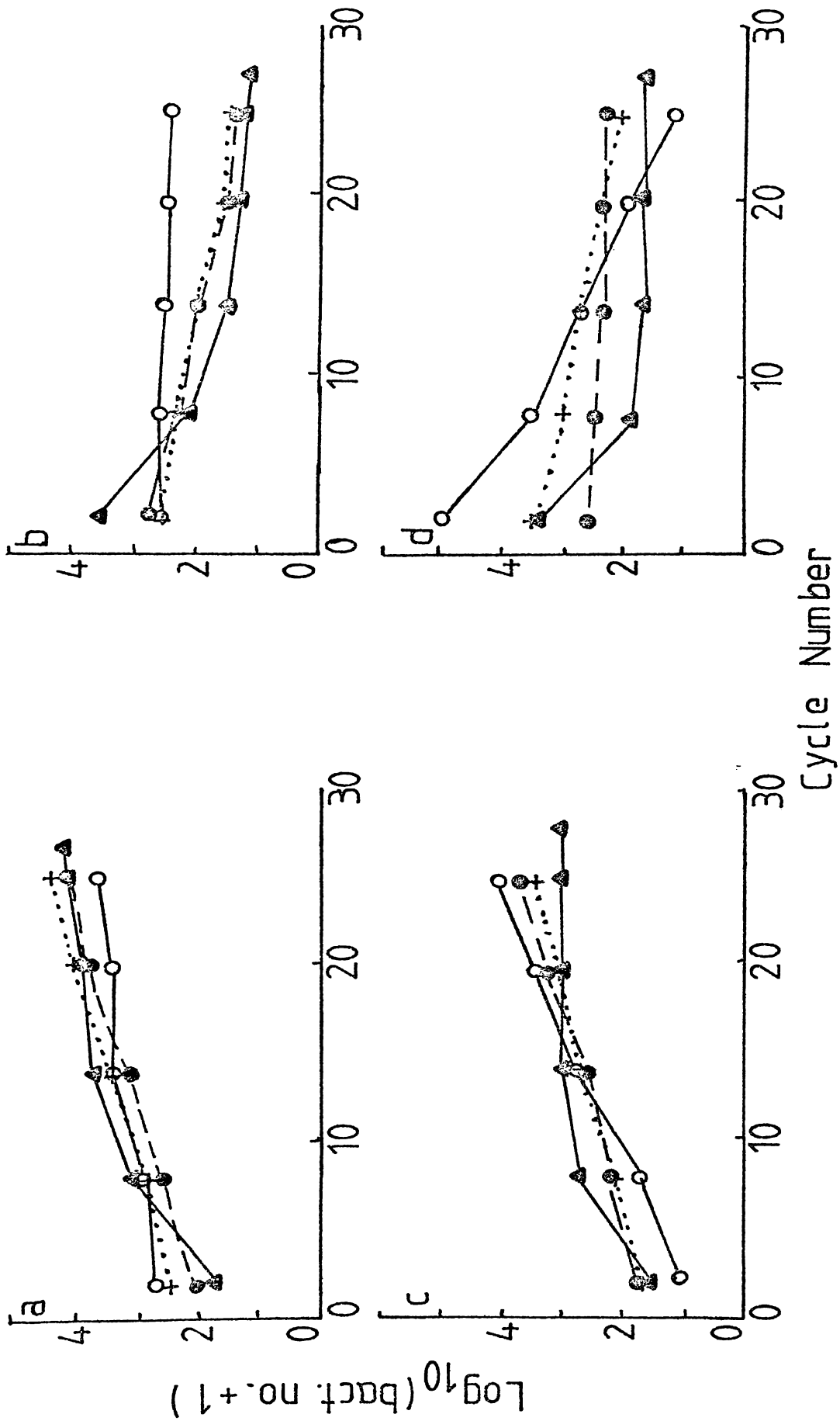

a
b
c
d
0
2
4
0
10
20
30
Log10(bact. no.+1)
Cycle Number

Figure 2.4-9: Influence of cleaning system on the effect of the final solution on bacterial numbers on stainless steel surfaces

(a) S. faecalis surviving final solution

(b) Decrease in S. faecalis with final solution

(c) E. coli surviving final solution

(d) Decrease in E. coli with final solution

Key: System C.1 (periodical acid)

System C.2 (no acid)

System C.3 (all acid)

System C.4 (iodophor)

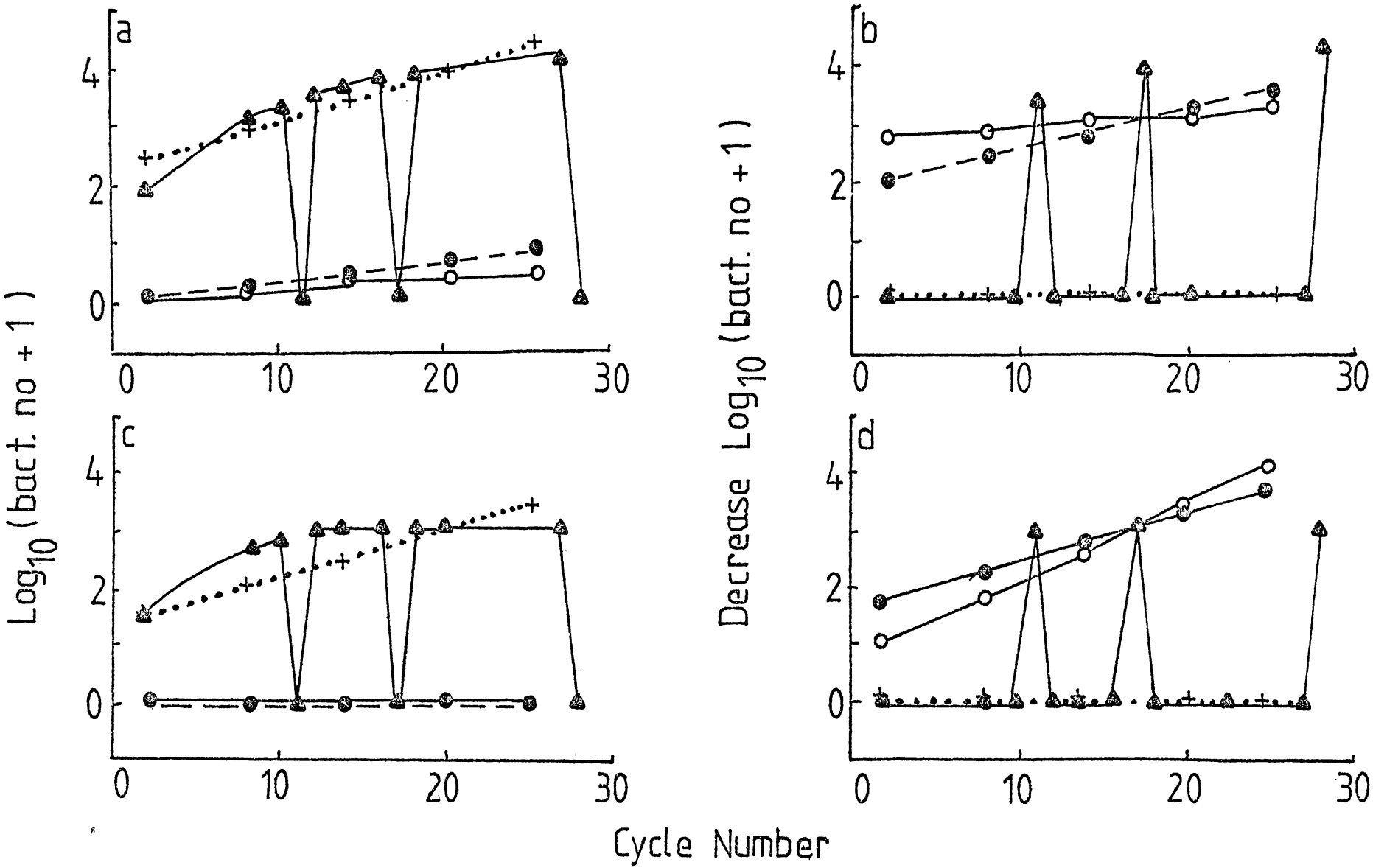

a
b
c
d
Log10 (bact. no + 1)
Decrease Log10 (bact. no + 1)
Cycle Number
0
2
4
0
10
20
30

Figure 2.4-10: Influence of the cleaning system on the effect of the intercycle period on bacterial numbers on stainless steel surfaces.

(a) S. faecalis surviving intercycle period

(b) Decrease in S. faecalis during intercycle period.

(c) E. coli surviving intercycle period

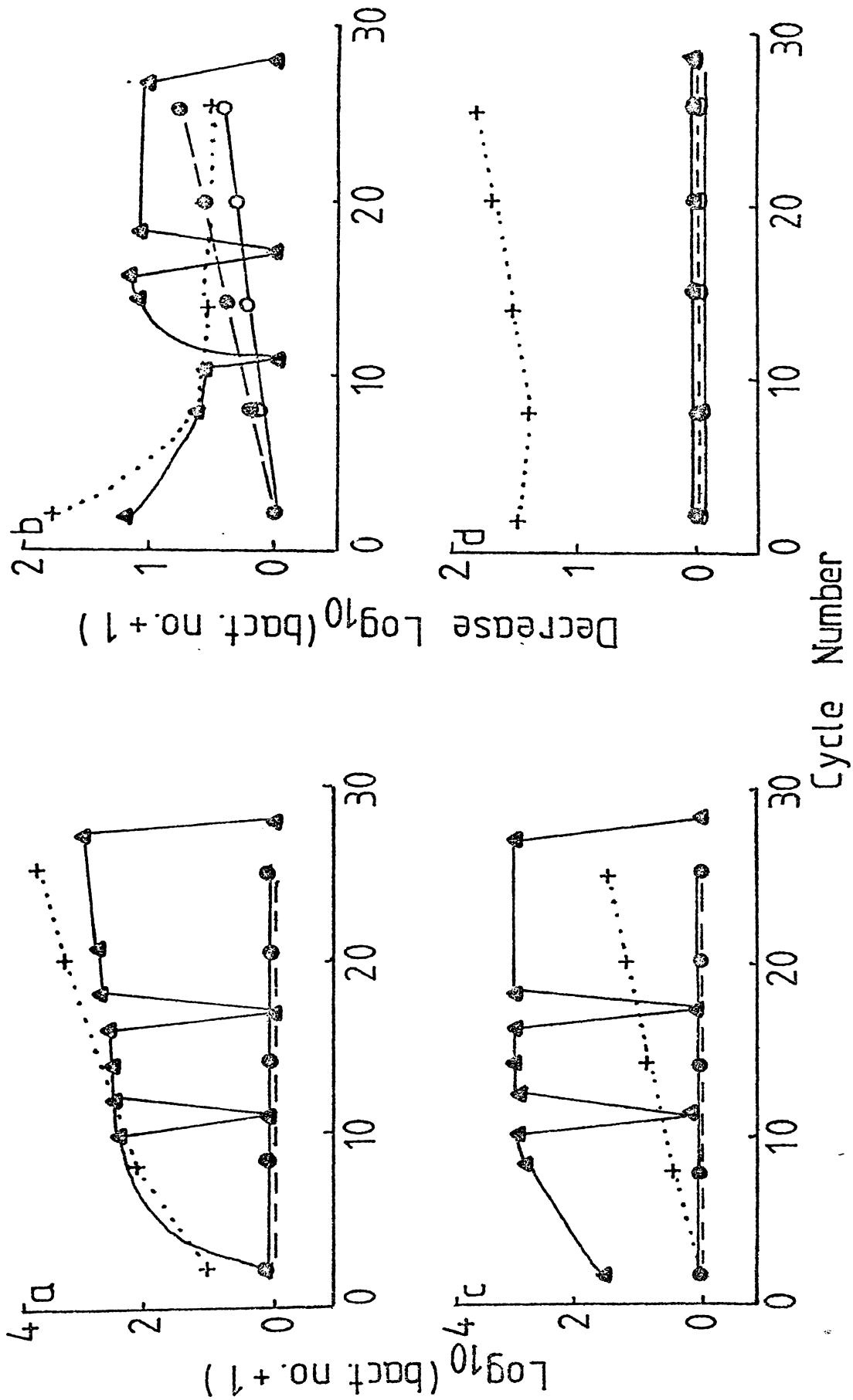
(d) Decrease in E. coli during intercycle period

Key: System C.1 (periodical acid)

System C.2 (no acid)

System C.3 (all acid)

System C.4 (iodophor)


a
b
c
d
Log10 (bact no. + 1)
Decrease Log10 (bact no. + 1)
Cycle Number
0
10
20
30
0
1
2
4

final solution, and seemed little affected by the soil levels with the different treatments (Figure 2.4-1a).

The data in Figure 2.4-6 show that on rubber surfaces the iodophor system (C.4) was superior to System C.3 (all acid) which was, in turn, superior to System C.2 (no-acid). As on the steel surfaces, this reflected the relative efficiencies of the final solution, and not the relative amounts of soil on the slide surfaces (Figure 2.4-1b).

The microflora of the steel surfaces following treatment with System C.2 (no acid) was quite varied (Table 2.4-2). It comprised a few of the dosing organisms: streptococci and coliforms; a group derived from the contaminated rinse water - flavobacteria, acinetobacter, pseudomonads, enterics other than coliforms, and a group of thermoduric organisms derived from the pasteurised milk used for soiling consisting of bacilli, micrococci and coryneforms. The microflora of the steel surfaces following treatment with System C.1 (periodical acid) was similar; except that it was dominated by the dosed streptococci without coliforms, it had no pseudomonads or coryneforms, but a few lactobacilli. No bacteria were present on the steel slides following use of Systems C.3 and C.4 (all-acid and iodophor systems).

The microflora of the rubber surfaces with System C.2 (no acid) also contained some of the dosed streptococci and coliforms, a number of rinse water contaminants - acinetobacter, pseudomonads, flavobacteria and non-coliform enterics, and many of both thermoduric bacilli and micrococci. System C.1 (periodical acid) gave a similar microflora, with fewer coliforms and no pseudomonads. With the frequent acid rinsing in System C.3 (all acid) coliforms

predominated, and acinetobacter and non-coliform enterics were the only rinse-water contaminants to survive. The iodophor final rinse of the iodophor System (C.4) killed all of the dosed organisms leaving only a few micrococci.

PERFORMANCE OF SYSTEM COMPONENTS

Milk Soiling

The numbers of S. faecalis and E. coli on the steel surfaces gradually increased linearly with time, with no significant differences between treatments (Figure 2.4-7). The numbers attaching at any time reflected both the amount of soil on the surfaces and the variable number of organisms on the surfaces immediately before soiling. This latter effect was particularly marked in those cycles of System C.1 (periodical acid) system in which the acid rinse was applied.

Effect of Detergent and Rinse

The reduction in count achieved by the detergent was considerable in early cycles, but diminished over time (Figure 2.4-8). The detergent affected S. faecalis similarly over all treatments, but its efficiency against E. coli in the early cycles of the iodophor system exceeded the other systems.

Effect of the Final Solution

The surface counts after treatment with the final solution reflected the bactericidal performance of the relevant solutions. The final rinse of water in the System C.2 (no acid) did not affect the surface counts of S. faecalis or E. coli at all. The numbers of survivors in the non-acid cycles of the periodical-acid system (C.1) were very similar to those of System C.2 (no acid). However, in

Figure 2.4-11: Influence of four cleaning systems on the bacterial numbers on stainless steel surfaces in complementary cleaning study

(a) S. faecalis surviving system

(b) Decrease in S. faecalis by total system

(c) E. coli surviving system

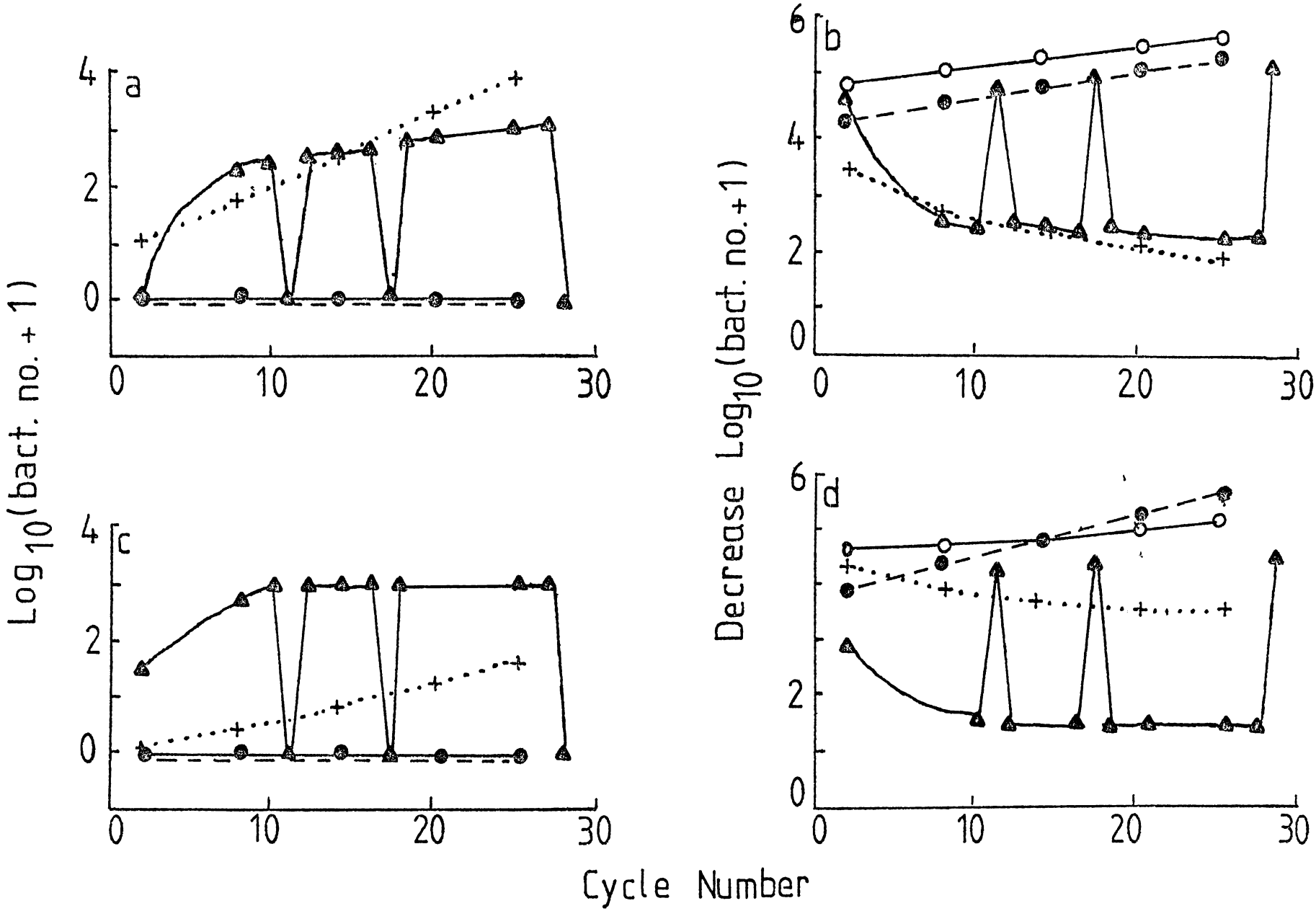
(d) Decrease in E. coli by total system

Key: System C.1 (periodical acid)

System C.2 (no acid)

System C.3 (all acid)

System C.4 (iodophor)


a
b
c
d
Log10(bact. no. + 1)
Decrease Log10(bact. no.+1)
Cycle Number
0
2
4
6
10
20
30

those cycles where the acid rinse was applied the numbers of viable S. faecalis and E. coli on the surfaces was zero.

The acidic detergent and iodophor final rinses of Systems C.3 and C.4 were both very effective in bacterial control. As the only difference in formulation between the iodophor and the acidic detergent was the added iodine, the very similar performance indicated that the acid contributed a significant portion of the bactericidal action of the iodophor. Any additional bactericidal effect of the iodine was not demonstrated under the conditions of this experiment.

Effect of Intercycle Time

The S. faecalis numbers diminished slightly during the intercycle period with all systems. The increasing decline in Systems C.3 and C.4 was due to an increasing number of organisms being present at the start of the intercycle period. The intercycle period caused a significant decrease in System C.2 (no acid). However, in Systems C3 and C.4 (all acid and iodophor) there were no viable coliforms at the commencement of the intercycle period.

EFFECT OF THE SUBSTRATES

The manner in which the soil accumulated was greatly influenced by the substrate material. The absolute levels of soil mass differed greatly - the soil on the rubber varied from 10 to 30 times that on the steel surfaces for the same cleaning system in this experiment (Figure 2.4-1a,-1b). The relative rates of soil accumulation also varied widely on the two surfaces. Further, the relative performance of different cleaning systems altered from one surface to another, the ranking of the amounts of deposit on steel being; System C.4 > System C.3 > Systems C.1, C.2, while that on rubber was; System C.3

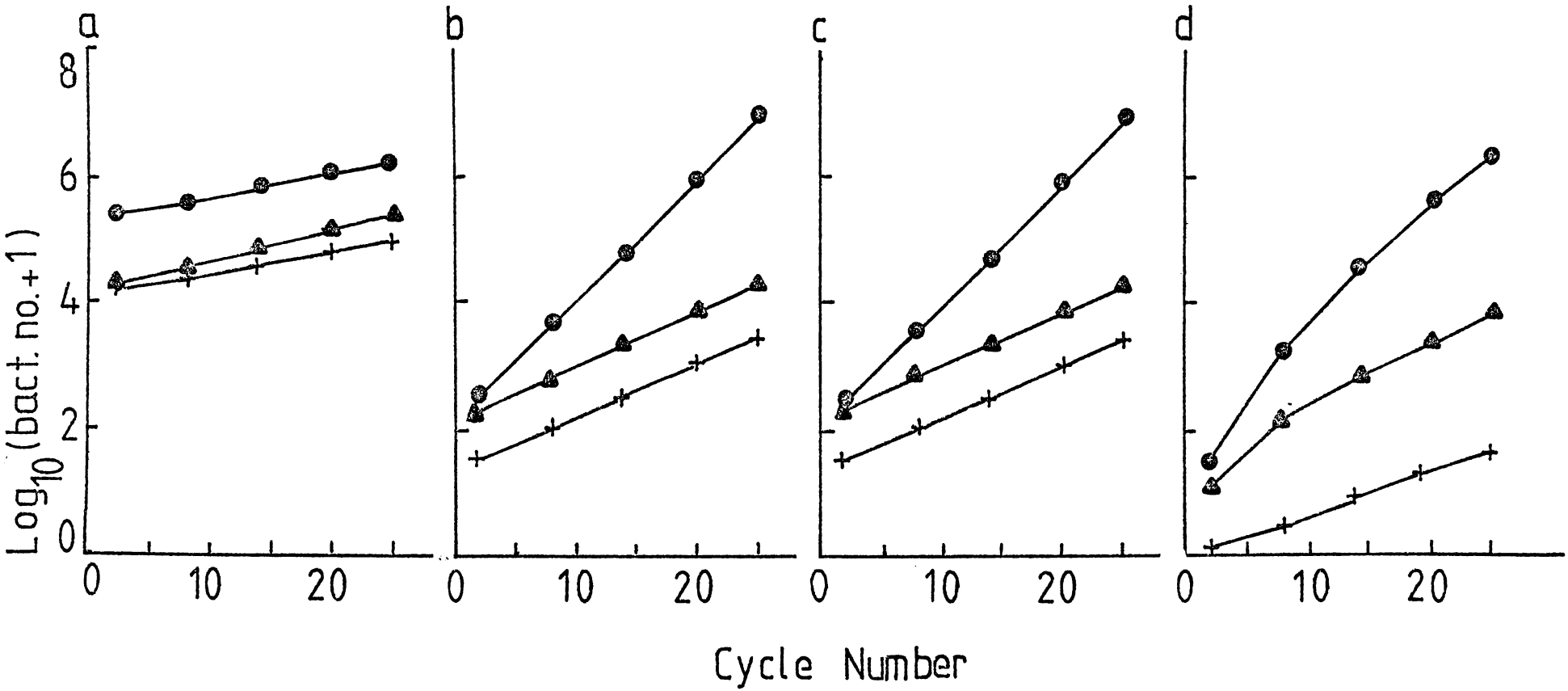
Figure 2.4-12: Changes in the relative numbers of three groups of bacteria on stainless steel slides washed by System C.2 (no acid) in complementary cleaning study.

(a) After milk soiling (b) After detergent and rinse (c) after final solution (d) After intercycle period

Key: Total count

Number of S. faecalis

Number of E. coli


a
b
c
d
8
6
4
2
0
0
10
20
Log_{10} (bact. no. + 1)
Cycle Number

> System C.4 > System C.2.

In those systems where growth occurred on the intercycle samples (Figures 2.4-6a,-6b), the surface bacterial counts on the rubber were far greater than on the steel (approximately double the $\log_{10}$ counts); this is expected considering the far greater amount of soil on these surfaces. The microflora on the two surfaces was very similar in both System C.2 (no acid) and System C.1 (periodical acid), the only systems for which data on both surfaces are available.

MICRO-ECOLOGY OF THE SURFACES.

The dosed organisms soiled the surfaces in equal numbers (Figures 2.4-12a to 12d), but S. faecalis survived the detergent slightly more successfully than did E. coli. In System C.2 (no acid) there were a large number of non-dosed organisms which did not survive as well in the other treatments where acidic rinses were applied. The final solution did not alter the relative order of the organisms.

Although the relative humidity was increased to 90% for this experiment (cf 80 and 85% for the previous two experiments), there was still no growth of either dosed organism in the intercycle period. Even in System C.2 (no acid), where there was a significant soil level, and no detergent or sanitizer residues on the surfaces for the intercycle period, neither S. faecalis nor E. coli grew during this time.

The dose organisms differed in their ability to survive the detergent washing and the intercycle rest period - S. faecalis was more resistant to both of these treatments (Figure 2.4-12). The proportion of the total organisms contributed by the dose organisms was affected by the detergent; non-dosed organisms being more

Table 2.4-2: Relative proportions of dominant organisms in microflora of rubber and stainless steel surfaces cleaned by 4 systems

Organisms	Substrate	Systems													
		C.1 (periodical acid)					C.2 (no acid)				C.3(all acid)		C.4(iodophor)		
		Cycle number					Cycle number				Cycle number		Cycle Number		
		10	11A*	16	17A*	27	2	10	16	27	16	27	2		
Streptococcus spp.	Steel	24	20	28	95	11			4						
	Rubber				15	9	100		4	3	3				
Bacillus spp.	Steel	43	8	-	-	6			32	17					
	Rubber								32	37	19	100			
Micrococcaceae	Steel	9	-	18	5	-			21	53					
	Rubber				12	-			21	37	6		100		
Coryneforms	Steel									27					
	Rubber														
Lactobacillus spp.	Steel			4											
	Rubber														
Pseudomonas spp.	Steel														
	Rubber								7	3					
Coliforms	Steel							11	11						
	Rubber								11	-	47				
Non-coliform enterics	Steel	19	56	45	-	72		6	4						
	Rubber				27	26			4	3	22				
Acinetobacter	Steel	5	12	-	-	11		6	14						
	Rubber								14	-	3				
Flavobacterium spp.	Steel			9				41	7	3					
	Rubber				46	65			7	17					
Total no. cultures	Steel	21	25	11	20	18		17	28	30					
	Rubber				13	23	10		28	30	32	8	10		

* Cycles at which periodical acid was applied

successful in surviving this treatment. The major members of the non-dosed microflora were Bacillus spp., micrococci and the non-coliform enterics. The first two of these were probably derived from the thermoduric population in the pasteurised milk used for soiling, and the last group from contaminated rinse water.

SUMMARY

The experiment has demonstrated that:

1. Complementary use of alkaline and acidic detergents does not work in the soak-cleaning situation as acidic detergents aggravate the accumulation of soil under these circumstances;
2. A considerable proportion of the bactericidal performance of an iodophor was contributed by the acidic-detergent fraction;
3. Substrates varied greatly in the absolute and relative amounts of soil which were deposited upon them - these differences affected the numbers of bacteria which the surfaces supported;
4. Although the periodic application of a sanitizer did not influence soil accumulation, it transiently affected the numbers and types of organisms on the surfaces.
5. As in the previous experiment, sanitizers applied as post-wash solutions were effective in bacteriological control;
6. The efficiency in bacterial control of the four systems was related not to the detergency, but to the performance of the sanitizer used in the system; and
7. Neither S. faecalis nor E. coli grew during the intercycle period of any system.

2.5 SIMULATOR COMPARISON OF PHYSICAL AND CHEMICAL SANITIZING

Since milking machines were first introduced they have been sanitized by heat in the form of hot water or steam. Later,cold chemical sanitizers were used in systems where a hot detergent was still applied. Thiel et al.(1955) introduced the cold detergent-sanitizer system (immersion cleaning) for the "direct-to-can" type of machine. Application of this type of system was only moderately successful in pipeline machines (Egdell & Widdas 1964). The recent energy crisis and also the spread of intensive dairying to under-developed countries have both created a need to eliminate the need for thermal energy in cleaning systems.

The following experiment was performed to:

1. Compare the performance of two heat-sanitizing (acidified boiling water system and the triple system) and two chemical-sanitizing systems (cold iodophor system and cold alkali system);
2. Investigate the influence of stainless steel, rubber and glass substrates on cleaning performance;
3. Examine the nature of the soil and bacteria on the surfaces of stainless steel, rubber and glass slides by scanning electron microscopy;
4. Provide information on the effect of the physical application method of cleaning solutions;by comparing the above four cleaning systems applied by soak cleaning techniques in the simulator with a later field study of the same systems applied by flow techniques in milking machines (Section 2.6).

2.5.1 EXPERIMENTAL DESIGN

The details of the 4 systems examined in the experiment are

Table 2.5-1: Components of 4 cleaning systems and sampling regimes in simulator comparison of physical and chemical sanitizing

Cleaning system		Components of cleaning system					
Number	Name	Pre-milk	Milk	Post-milk rinse	Detergent	Final rinse	Intercycle
D.1	Acidified boiling water (ABW)	-	+	-	Sulphamic acid, 1.5 g/l, 85°C	Water, 85°C	-
D.2	Triple (T)	Iodophor, 1.5 ml/l, 20°C	+	Nonionic, 2.5 ml/l, 20°C	Heavy duty alkaline detergent, 3.0 ml/l, 75°C	Water, 85°C	-
D.3	Cold iodophor (CI)	Chlorhexidine, 3.0 ml/l, 20°C	+	Nonionic, 2.5 ml/l, 20°C	Iodophor/acid, 4.0 ml/l, 20°	-	-
D.4	Cold alkali (CA)	Iodophor, 1.5 ml/l, 20°C	+	Nonionic, 2.5 ml/l, 20°C	Chlorinated alkali detergent, 5.0 g/l, 20°C	-	-

Sampling times:

- Every sampling cycle;
  - gravimetric — G
- Final cycle only;
  - Surface bact. no.
    - steel — I, S, M, R, D, F
    - rubber — RS, RM
    - glass — GS, GM
  - Contamination bact no. - steel, rubber, glass — (CS,CR,CG)
  - Microflora - steel, rubber, glass — (MS,MR,MG)
  - SEM - steel — SI, S, S, SM, SR, SD, SF
    - rubber — SR
    - glass — SG

presented in Table 2.5-1. System D.1 (acidified boiling water - ABW) is a two-component hot-acid system developed in the UK (Clough et al. 1967). The triple system (D.2) is a hot-alkaline system with four components developed in Australia (Calder & Twomey, 1966). The cold iodophor system (D.3) is a cold-acid system with three components developed in Australia (Dunsmore, unpublished). The cold alkali system (D.4) has three components and had not been previously evaluated.

Crystalline sulphamic acid at 1.5 g/l was used as the detergent in System D.1. The iodophor used for the pre-milking sanitizer in systems D.2 and D.4 was "Iodophor" supplied by Wellcome NZ Ltd, and applied at 1.5 ml/l (25 mg Av. I/l). The iodophor/acid used as the detergent in System D.3 was the iodophor "2-4 6" supplied by Wellcome NZ Ltd, mixed 2:1 with H_3PO_4 (technical grade), with this mixture applied at a concentration of 4.0 ml/l (45 mg Av. I/l). The nonionic rinse-additive used in the post-milking rinse in systems D.2, D.3 and D.4 was "Coopersol D" (a nonyl phenol polyethylene oxide condensate) supplied by Wellcome NZ Ltd and applied at a concentration of 2.5 ml/l. The heavy-duty alkaline detergent used in System D.2 was a caustic-based liquid "Det-Flo" supplied by Wellcome NZ Ltd and applied at a concentration of 3.0 ml/l. The chlorhexidine used as a pre-milking sanitizer in System D.3 was "Hibitane" supplied by I.C.I.A.N.Z. Ltd and applied at 3 ml/l (30 mg chlorhexidine/l). The chlorinated-alkaline detergent used in System D.4 was "AL-1" supplied by Alfa-Laval NZ and applied at a rate of 5 g/l (200 mg Av. Cl/l).

The experimental treatments were applied for 36 cycles. Triplicate stainless steel, rubber and glass slides were removed for gravimetric assessment of soil deposition immediately before the milk

Figure 2.5-1: Changes in soil mass on three surfaces treated with 4 cleaning systems over 36 cycles

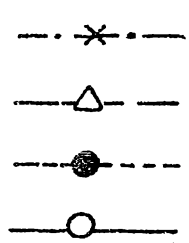
(a) Stainless steel

Key: System D.1 (acidified boiling water)

System D.2 (triple)

System D.3 (cold iodophor)

System D.4 (cold alkali)



(b) Rubber

Key: as for 2.5-1a

(c) Glass

Key: as for 2.5-1a

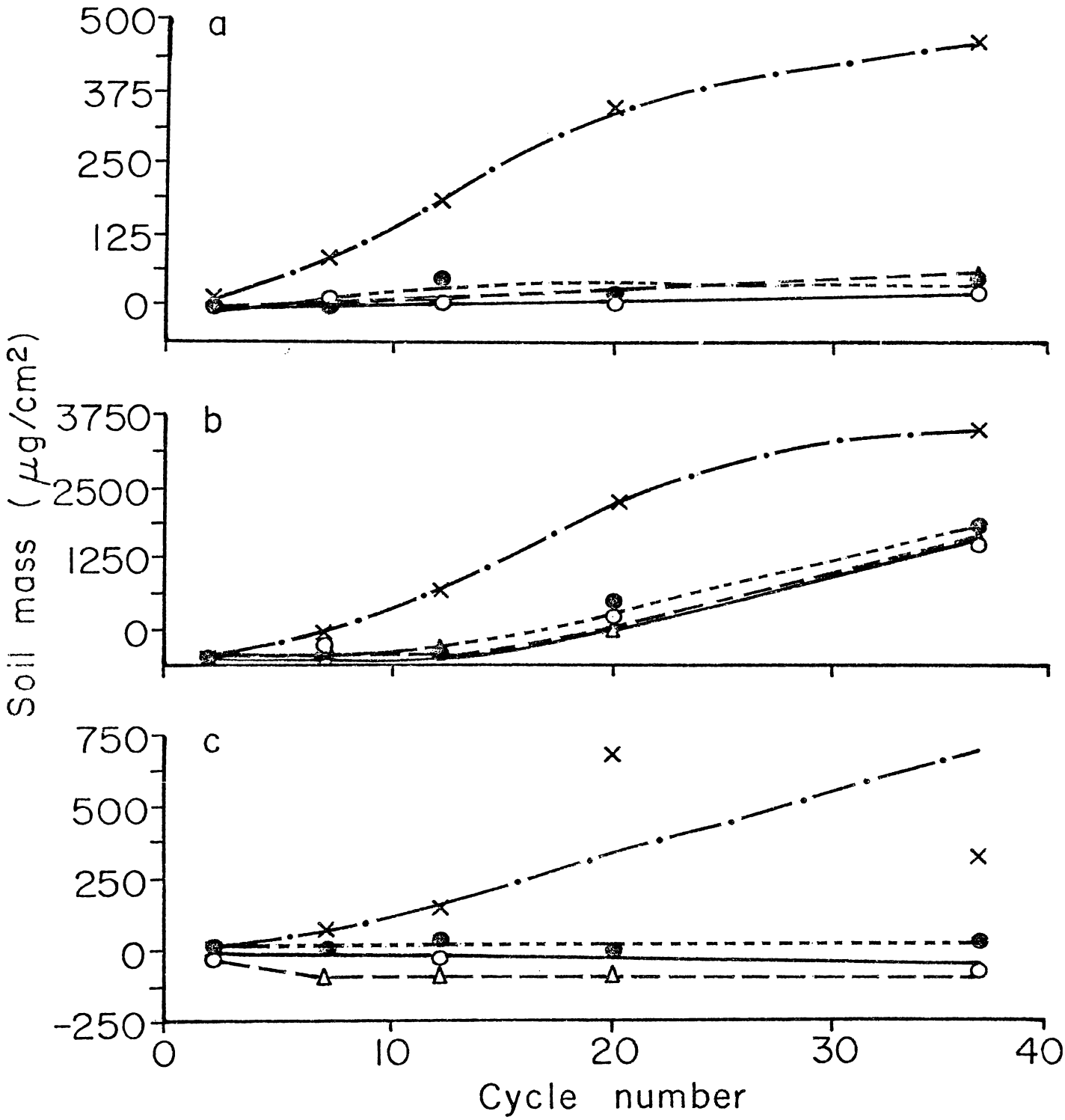

a
b
c
500
375
250
125
0
3750
2500
1250
0
750
500
250
0
-250
0
10
20
30
40
Soil mass (μg/cm2)
Cycle number

Table 2.5-2: Intercycle, contamination and milk-soiling bacterial counts ($\log_{10}$ (x + 1)) after 36 cycles on three surfaces in the study of physical and chemical sanitizing

Organism	Cleaning system	Surface material								
		Steel			Rubber			Glass		
		* Int.	** Cont.	Milk soil	* Int.	** Cont.	Milk soil	* Int.	** Cont.	Milk soil
S. faecalis	D.1 (ABW)	0.00	0.00	2.47	0.00	0.00	2.63	0.00	0.00	3.00
	D.2 (T)	0.00	0.00	2.28	0.00	0.00	3.98	0.00	0.00	4.51
	D.3 (CI)	0.00	0.00	4.92	1.69	0.00	4.90	1.88	0.96	4.97
	D.4 (CA)	0.00	0.00	4.04	0.00	0.00	4.13	0.00	0.00	4.34
	Surface mean	0.00	0.00	3.93	0.42	0.00	3.91	0.47	0.24	4.21
E. coli	D.1 (ABW)	0.00	0.00	2.98	0.00	0.00	3.12	0.00	0.00	0.83
	D.2 (T)	0.00	0.00	2.24	0.00	0.00	4.11	0.00	0.00	5.07
	D.3 (CI)	0.00	0.00	4.97	1.53	0.80	4.88	2.33	1.43	4.63
	D.4 (CA)	0.00	0.00	3.98	0.00	0.00	4.35	0.00	0.00	4.06
	Surface mean	0.00	0.00	3.54	0.38	0.20	4.12	0.58	0.36	3.65
Total	D.1 (ABW)	0.00	0.00	3.83	1.55	0.47	3.70	0.00	0.00	3.60
	D.2 (T)	0.93	0.00	6.13	0.00	0.00	6.32	0.60	0.00	7.00
	D.3 (CI)	0.00	0.00	6.58	1.65	1.25	6.74	2.49	2.91	6.76
	D.4 (CA)	0.62	0.00	5.69	0.90	0.00	6.98	0.00	0.00	4.90
	Surface mean	0.41	0.00	5.56	1.05	0.43	5.94	0.77	0.73	5.57

* Count on surface at end of intercycle period.

** Number of organisms on surface before milk soiling recovered by fluid rinsing.

Figure 2.5-2: Changes in the numbers of 3 groups of organisms through the 36th cycle on stainless steel surfaces treated with 4 systems

(a) S. faecalis

(b) E. coli

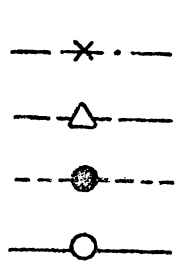
(c) Total

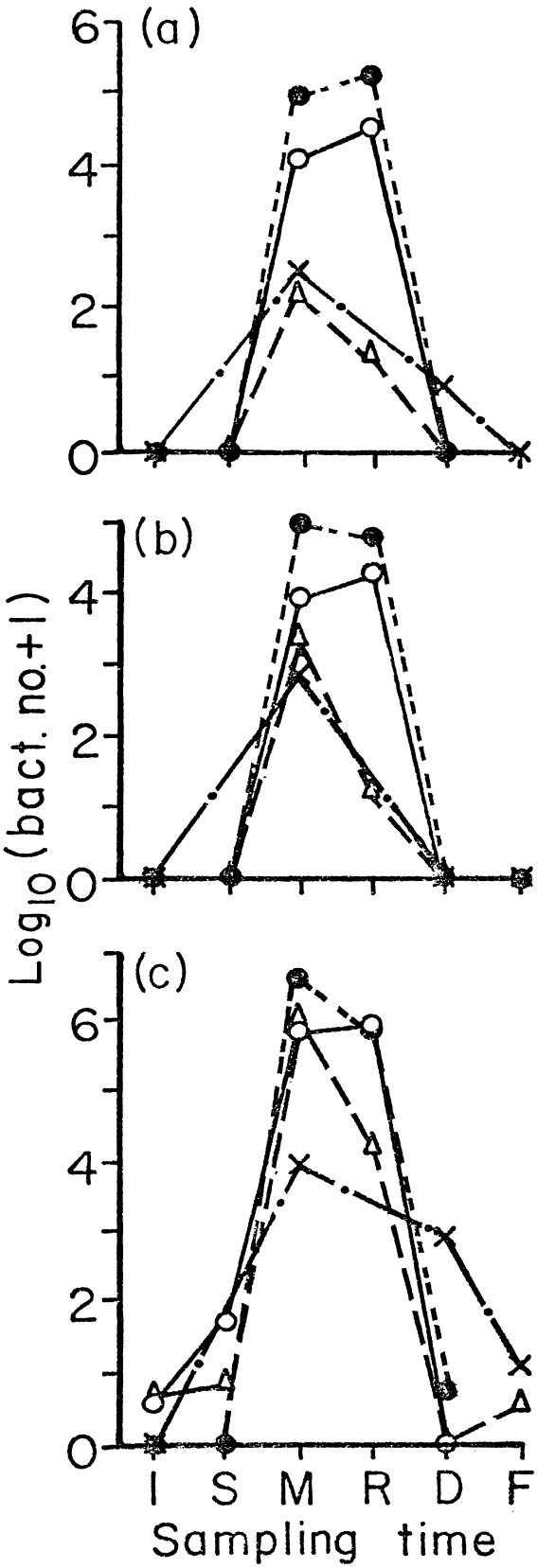
Key: System D.1 (Acidified boiling water)

System D.2 (triple)

System D.3 (cold iodophor)

System D.4 (cold alkali)




(a)
(b)
(c)
6
4
2
0
Log10 (bact. no.+1)
I S M R D F
Sampling time

soiling in cycles 2, 7, 12, 20 and 36 (G in Table 2.5-1).

During the 36th cycle stainless steel slides were removed in triplicate at the times indicated in Table 2.5-1 (after intercycle - I, after pre-milk sanitizer - S, after milk soil - M, after post-milk rinse - R, after detergent - D and after the final rinse - F). Rubber and glass slides were removed in triplicate after the inter-cycle period and after the milk soiling only (RS, GS and RM, GM respectively). Single steel pieces were removed for SEM examination at the same times as the steel for surface bacterial count (SI, SS, SM, SR, SD and SF) while rubber and glass samples for SEM examination were taken after the pre-milk sanitizer only (SR and SG respectively). Also, after the pre-milk sanitizer,one slide of each of the three materials was removed for examination of the microflora (MS, MR, MG). Slides of each material were removed for enumeration of the "contamination" bacteria (CS, CR, CG - Section 2.1-7).

The test cells were maintained at a relative humidity of 95%. The dosing organisms S. faecalis and E. coli were each used at a rate of 5×10^5/ml to give a combined concentration of 10^6 organisms/ml in the soiling milk.

2.5.2 RESULTS

The changes in soil mass with the four systems on the three surfaces are presented in Figure 2.5-1. On each substrate the soil accumulation was much greater with System D.1 (ABW) than with the cold-iodophor system (D.3), which was only marginally greater than both the triple (D.2) and cold-alkaline systems (D.4).

The surface counts after the intercycle period, milk soiling, and for contamination on the three substrates during cycle 36 are

Table 2.5-3: Classification of dominant organisms in microflora of 3 surfaces after 36 cycles of 4 cleaning systems in comparison of physical and chemical sanitizing

System	Substrate	Proportion of population (%)						Total number samples
		Micrococcaceae	Streptococcus spp.	Flavobacterium spp.	Acinetobacter	Non-coliform enterics	Pseudomonas spp.	
D.1 ABW	Steel	93	7					14
	Rubber			60	3	7	30	30
D.2 Triple	Glass			25		75		4
D.3 CI	Glass	G		100				4
D.4 CA	Steel	27				13	60	30
	Rubber	10	3	3		3	80	30
	Glass	100						5

Figure 2.5-3: Scanning electron micrographs of stainless steel surfaces after 36 cycles of treatment by System D.1 (acidified boiling water).

(a) After intercycle period (magnification 2,000X) Evenly distributed soil of small particle size with two cocci.

(b) After milk soiling (magnification 5,000X) Evenly distributed soil of larger particle size after milk soiling, with two cocci covered by a thin layer of soil.

(c) After detergent (magnification 5,000X) Large particle-size soil, the amount reduced by the detergent, with a rod-shaped organism attached to the steel surface.

(d) After final rinse (magnification 3,900X) Evenly distributed soil of smaller particle size with the protein denatured by the combined effect of heat and acid of detergent and final rinse.

Key: ⟵ is 4 μm long.

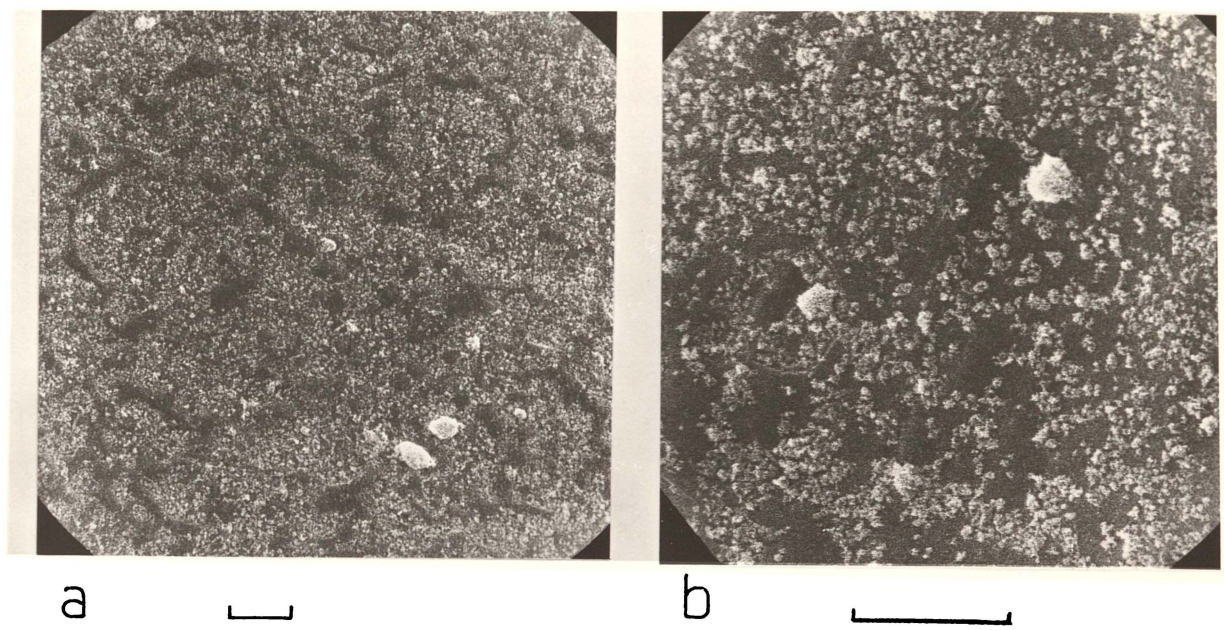

a
b
c
d

Figure 2.5-4: Scanning electron micrographs of stainless steel surfaces after 36 cycles of treatment by System D.2 (triple)

(a) After intercycle period (magnification 3,000X) Evenly distributed particulate soil with some aggregations.

(b) After milk soiling (magnification 3,000X) Voluminous spongy soil covering coccus (lower right) over particulate soil.

(c) After post-milk rinse (magnification 3,000X) Evenly distributed particulate soil.

(d) After detergent (magnification 3,000X) Evenly distributed particulate soil.

(e) After final rinse (magnification 3,000X) Evenly distributed particulate soil, with surface imperfection of steel substrate.

Key: ⌊⌋ is 1 μm long

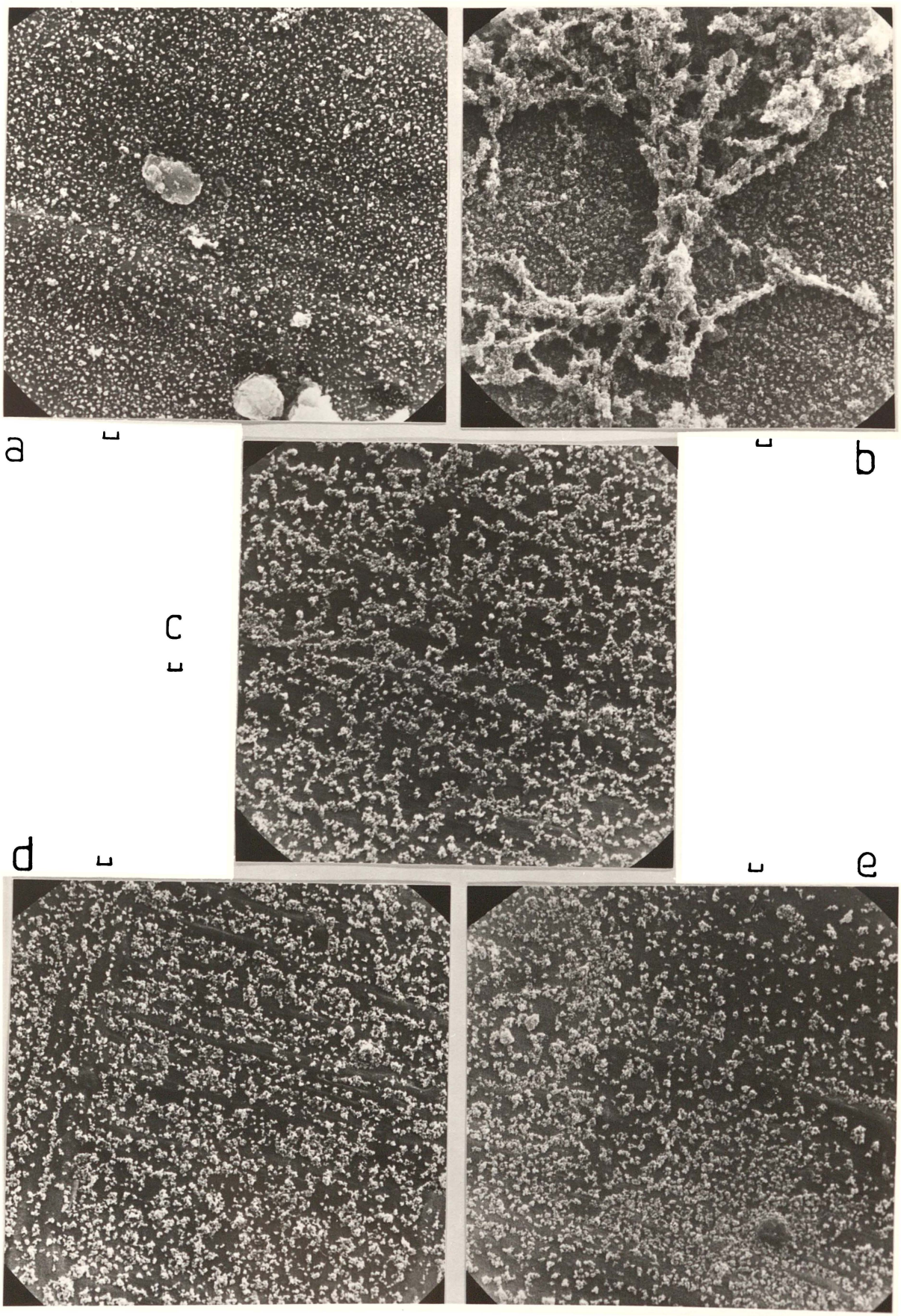

a
b
c
d
e

Figure 2.5-5: Scanning electron micrographs of stainless steel surfaces after 36 cycles of treatment by System D.3 (cold iodophor)

(a) After intercycle period (magnification 16,000X) Evenly distributed particulate soil with rod-shaped organisms attached to the steel substrate between particles.

(b) After sanitizing (magnification 3,000X) Evenly distributed particulate soil.

(c) After milk soiling (magnification 3,000X) Sheet of soil, breaking up at the top and right and covered with particulate soil and coccoid and rod-shaped organisms.

(d) After post-milk rinse (magnification 1,300X) Very spongy soil after rinsing containing coccoid organisms (centre right).

(e) After detergent (magnification 1,600X) Spongy soil with protein coagulated by acidity of iodophor detergent, coccoid organism partly buried (centre).

Key: ⌊⌋ is 2 μm long

Figure 2.5-6: Scanning electron micrographs of stainless steel surfaces after 36 cycles of treatment by System D.4 (cold alkali)

(a) After intercycle period (magnification 3,000X) Evenly distributed soil of small particles with some cocci.

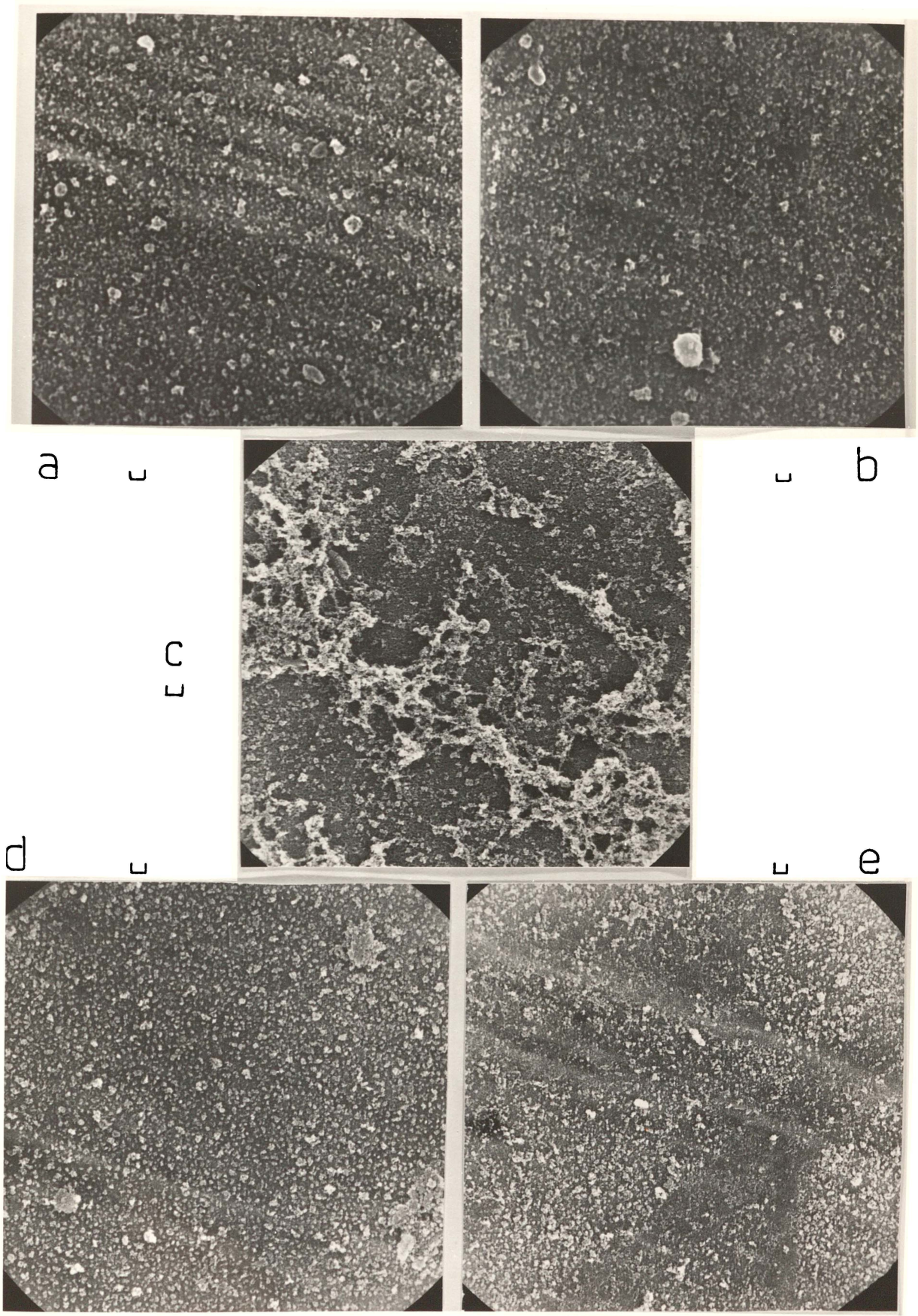
(b) After sanitizing (magnification 3,000X) Evenly distributed soil with a soil aggregate and some cocci.

(c) After milk soiling (magnification 3,000X) Voluminous spongy soil after milk soiling with a rod-shaped organism (upper left).

(d) After post-milk rinse (magnification 3,000X) Evenly distributed particulate soil with some cocci with soil particles attached.

(e) After detergent (magnification 3,000X) particulate soil.

Key: ⌊⌋ is 1 μm long


a
b
c
d
e

presented in Table 2.5-2. These show that for most systems on most substrates the intercycle or contamination counts were zero. The counts after milk soiling were similar for all substrates.

The change in numbers of viable organisms on steel surfaces during the 36th cycle are presented in Figure 2.5-2. Data on the microflora present on the three substrates after the intercycle period of the 36th cycle are presented in Table 2.5-3. As there were no viable organisms in many of the intercycle samples there is not a complete set of information on the microflora present for each substrate and cleaning system.

Scanning electron micrographs of the steel surfaces exposed to the four components of the acidified-boiling water system (D.1) and removed during the 36th cycle, are presented in Figure 2.5-3. Similar information for System D.2 (triple), D.3 (cold iodophor) and D.4 (cold alkali) are presented in Figures 2.5-4, 2.5-5 and 2.5-6 respectively. Micrographs taken at the intercycle period of the 36th cycle of each cleaning system are presented in Figure 2.5-7 for rubber surfaces and 2.5-8 for glass surfaces.

2.5.3 DISCUSSION

PERFORMANCE OF THE TOTAL SYSTEM

On all three surfaces the ABW system (D.1) permitted the heaviest soil deposition by far (Figure 2.5-1). The other systems all performed similarly to each other, with the cold iodophor system (D.3) permitting slightly greater deposit accumulation than the triple (D.2) and cold alkali system (D.4). Thus, the alkaline systems appear to provide better detergency than the acidic systems, which is in agreement with the results on acid rinses in Section 2.4.

Figure 2.5-7: Scanning electron micrographs of rubber surfaces during the intercycle period after treatment for 36 cycles by 4 cleaning systems

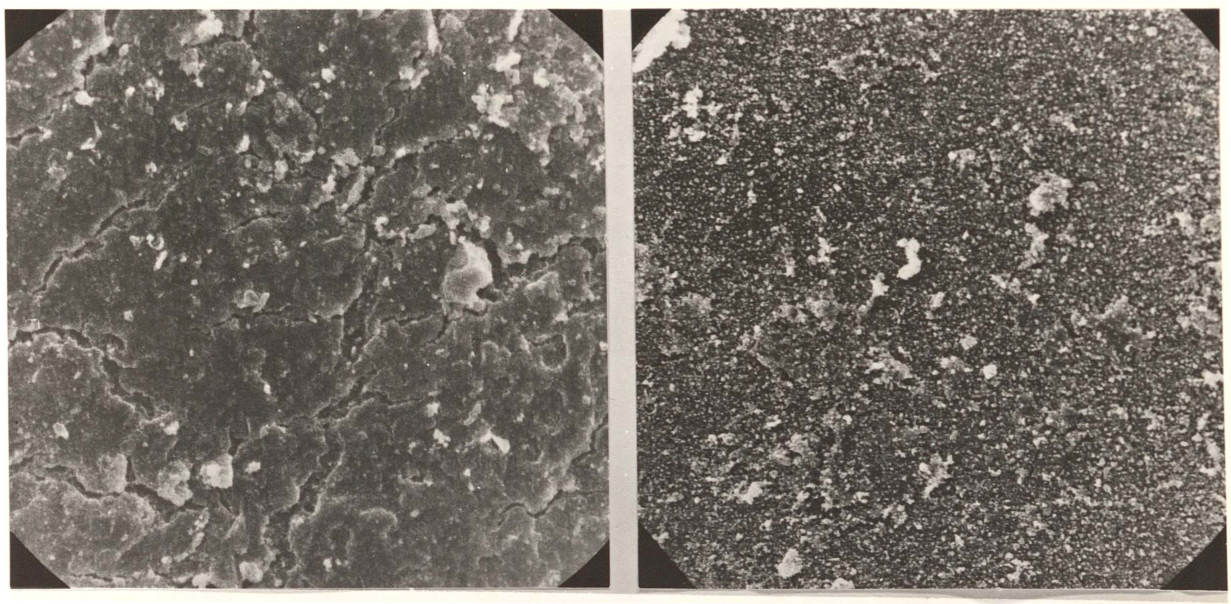
(a) System D.1 (Acidified boiling water - magnification 3,000X) Fractured rubber surface with unevenly distributed particulate soil.

(b) System D.2 (triple - magnification 1,000X) Entire rubber surface supporting unevenly distributed particulate soil, with coccoid and rod-shaped organisms.

(c) System D.3 (cold iodophor - magnification 1,500X) Variety of organisms, predominantly long rods, on even substrate.

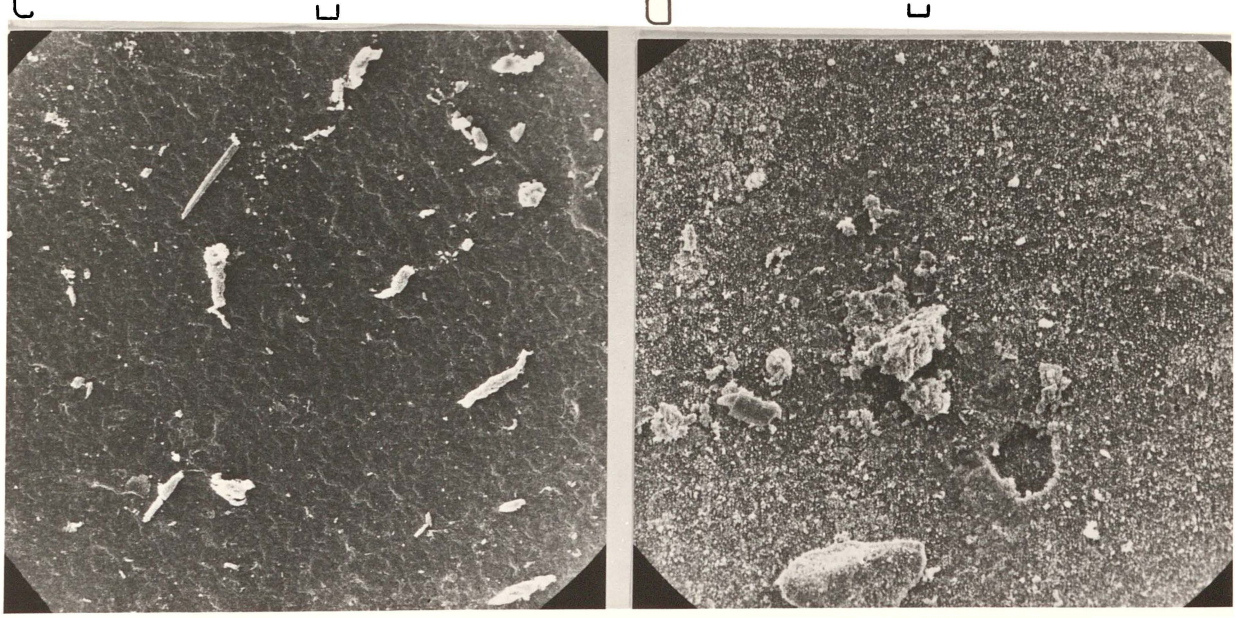
(d) System D.4 (cold alkali - magnification 1,000X) Rubber surface with local depression with particulate and aggregated soil, with some organisms.

Key: ⌊——⌋ is 1 μm long



a

b



c

d

Figure 2.5-8: Scanning electron micrographs of glass surfaces during the intercycle period after treatment for 36 cycles by 4 cleaning systems

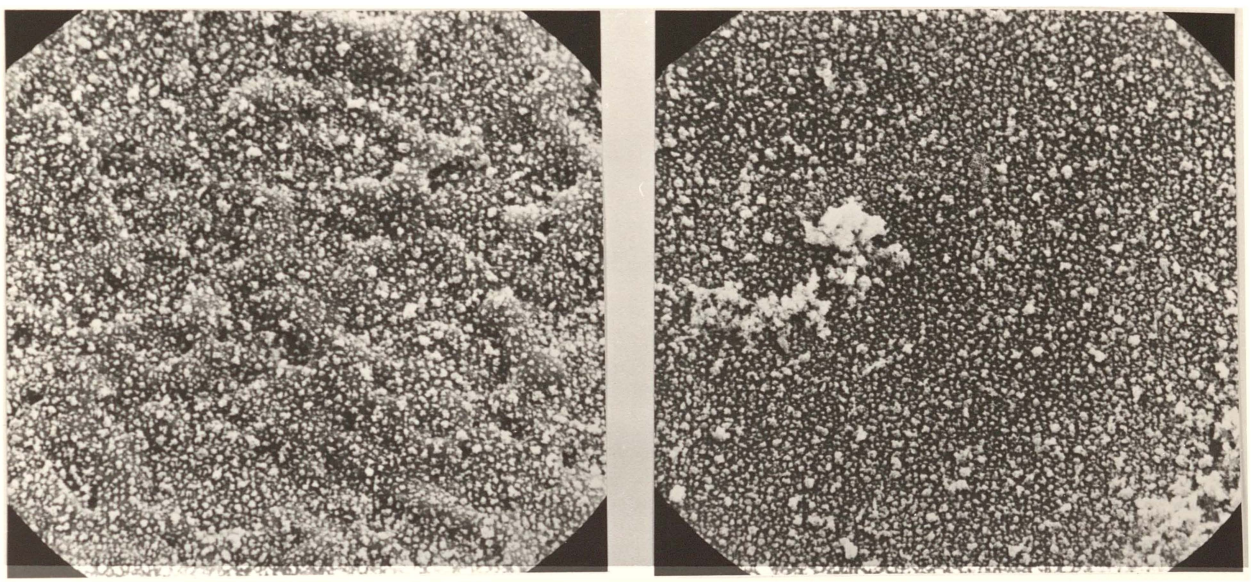
(a) System D.1 (Acidified boiling water - magnification 3,000X) Evenly distributed particulate soil on wavy substrate.

(b) System D.2 (triple - magnification 3,000X) Evenly distributed particulate soil with some soil aggregates on a planar substrate.

(c) System D.3 (cold iodophor - magnification 3,000X) Particulate soil distributed over wavy base soil layer.

(d) System D.4 (cold alkali - magnification 3,000X) Evenly distributed particulate soil with some coccoid organisms.

Key: ⌊——⌋ is 1 μm long

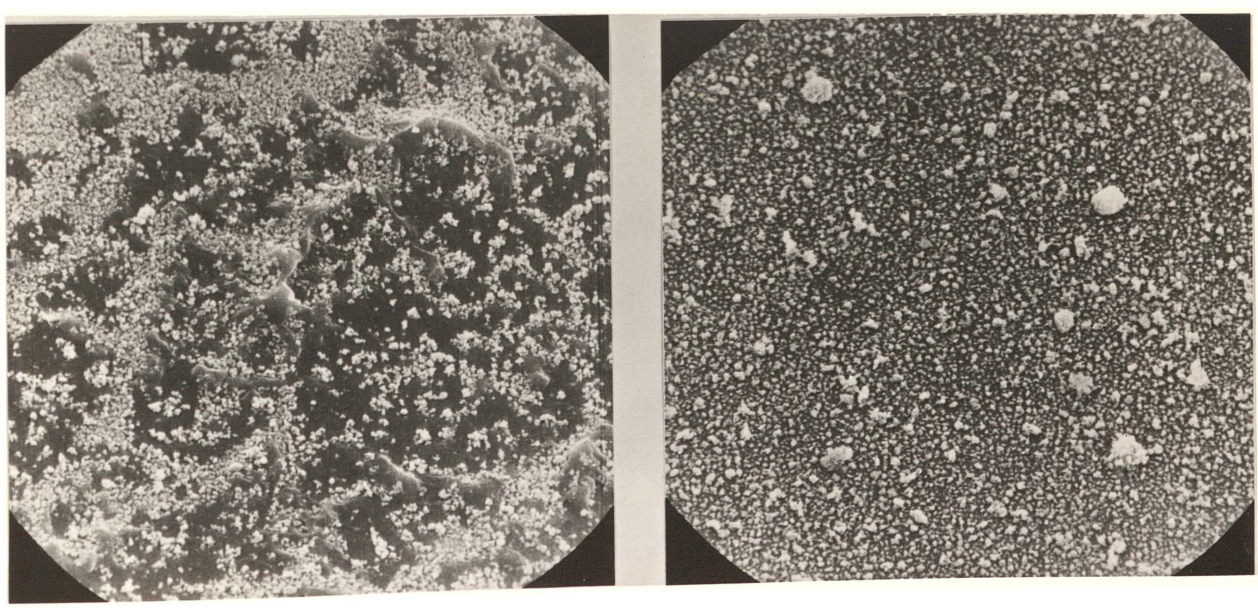


a

b

c

d



The bacteriological performance of a cleaning system is best measured by determining the number of viable organisms on a surface immediately before the milk soiling. Examination of the data in Table 2.5-2, shows that the surface intercycle counts are zero or very low and variable, which prevents their use as indices of performance in comparing the four systems. However, there is information on the numbers of organisms on the surfaces after the milk soiling which, although a slightly insensitive measure of system performance, provides the best data available on the four systems studied. The data for the total bacterial count after milk soiling showed that on all substrates the ABW system (D.1) had fewer bacteria on the surfaces than the cold-alkali system (D.4) which had fewer than both the triple (D.2) and cold-iodophor systems (D.3). These results show that, despite having the highest soil mass, the sanitizing by hot water in the ABW system was sufficient to provide very effective bacteriological control. The triple system (D.2) which had lower deposits than D.1 used slightly less heat and was much less efficient bacteriologically.

The changes in bacterial numbers on the surfaces of the steel slides during the 36th cycle are presented in Figure 2.5-2c. The scanning electron micrographs for the same period are presented in Figure 2.5-3. At the end of the intercycle period the surface was fairly evenly covered with a particulate soil (Figure 2.5-3a). There was a much greater deposit after the milk soiling (Figure 2.5-3b), which partly buried some cocci. The detergent removed most of the deposit leaving an even, particulate soil (Figure 2.5-3c). The heat of the detergent and final rinse (Fig 2.5-3d) changed the nature of the deposit, presumably by denaturation and dehydration of the

protein, and left few viable organisms on the surface.

At the end of the intercycle period with System D.2 (triple) there was an even, particulate soil (Figure 2.5-4a) which supported some bacteria (Figure 2.5-2c). The thick, spongy deposit which contained and covered bacteria after the milk-soiling (Figure 2.5-4b) was removed by the post-milk rinse, detergent, and final rinse of hot water. No viable organisms were observable after the detergent.

Following the intercycle period, the steel surfaces treated by System D.3 (cold iodophor) also had an evenly-distributed particulate soil with a few rod-shaped organisms attached to the substrate (Figure 2.5-5a). The milk soiling left a large sheet-like deposit which broke up in areas, and supported bacteria (Figure 2.5-5c). The post-milk rinse removed much of the soil (Figure 2.5-5d) and more was removed by the iodophor/acid detergent, leaving a network of soil, in which the proteins appear coagulated by the acid of the cleaning agent, and within which some organisms were buried (Figure 2.5-5e).

After the intercycle period in System D.4 (cold alkali - Figure 2.5-6a) the steel surfaces were covered with an even, particulate soil, with a few rods and cocci. This was not altered by the pre-milk sanitizer, and a voluminous deposit containing rods was laid down during milk soiling (Figure 2.5-6c). Most of the soil was removed by the rinse and detergent leaving a small amount of particulate soil evenly distributed, with few live bacteria (Figure 2.5-6e).

The contamination counts in Table 2.5-2 represent that proportion of the organisms present at the end of the intercycle period which could be moved from the surface into the milk at the next soiling, effecting contamination. These counts show that, on all surfaces

where the intercycle count ($\log_{10} x + 1$) was less than approximately 1.5, no contamination occurred. Where the count exceeded this level, contamination occurred, but insufficient data are available to determine what proportion of the organisms effected contamination.

COMPARISON OF SURFACES

The soil mass was approximately ten times greater on the rubber surfaces than both the glass and steel surfaces (Figure 2.5-1). The nature of the soil accumulation curves also differed with the substrate. For Systems D.2, D.3 and D.4 the deposit increased linearly on steel, but remained unchanged for many cycles on rubber and then increased linearly. For the same systems on glass a deposit level was established in the first few cycles which remained unchanged thereafter.

Scanning electron micrographs of the surfaces of stainless steel during the intercycle period with the 4 cleaning systems are presented in Figures 2.5-3a,-4a,-5b and-6b. Similar information for the rubber and glass surfaces is presented in Figures 2.5-7 and 2.5-8 respectively. The steel surfaces were entire and slightly wavy. The rubber surfaces were much rougher, providing sites for bacterial harbourage, such as surface cracks (2.5-7a) or local depressions (2.5-7d). The glass surfaces were very smooth with no observable imperfections (e.g. Figures 2.5-8b,-8d).

Despite the marked differences in soil masses there were no major differences between the bacterial populations on the surfaces of the different materials (Table 2.5-2). However, the microflora does alter with different surfaces. With the ABW system (D.1) micrococci dominated the steel surfaces while the rubber supported mainly flavobacteria and pseudomonads. With the cold-alkali system (D.4)

the steel and rubber supported similar microflora, primarily pseudomonads with some micrococci, while the glass supported only micrococci. The surface probably alters the chemical composition and amount of the soil which, in turn, influences the microflora in the soil.

ECOLOGY OF THE EQUIPMENT SURFACES

This experiment demonstrated the important interactions between the many factors in the microenvironment of the surface than can affect the survival of bacteria on that surface. The major factors are: the surface itself, the soil, the organisms, the physical conditions and the cleaning system. For example, although the surfaces of the rubber were degraded by the acidified-boiling-water system (D.4) permitting a large amount of soil to accumulate and providing protected sites for bacteria (Figure 2.5-7a), the heat sanitizing kept bacterial development to very low levels.

It is difficult to determine the exact nature of the relationship between bacteria, soil and substrate. Where soil levels are high, particularly after milk soiling, the bacteria may be submerged in the soil and protected by it from the environment (e.g. Figure 2.5-4b). However, after the cleaning system has been applied there appears to be a thin layer of particulate soil on the surfaces, the particles having a diameter of 0.1 - 0.3 um, and the bacteria appear to adhere directly to the substrate and not to the soil (e.g. Figures 2.5-5a and 2.5-7c). The bacteria are relatively widely dispersed as single organisms or pairs.

SUMMARY

The experiment has demonstrated:

1. That three of the four systems achieved satisfactory control of deposit accumulation, while the fourth (system D.1 - ABW) was unsatisfactory;
2. That both heat-sanitizing and chemical-sanitizing systems achieved satisfactory control of bacteria on the surfaces;
3. That the type of substrate influenced both the rate of soil deposition, and the absolute amount of soil deposited. These two factors may have, in turn, influenced the microflora of the soil; and
4. The relationships between bacteria and soil on three substrates by scanning electron microscopy.

2.6 FIELD COMPARISON OF PHYSICAL AND CHEMICAL SANITIZING

The solutions used to clean milking machines have been applied to the surfaces by flowing them through pipelines, brushing and immersing articles in static solutions. The simulator used in Sections 2.2 - 2.5 examined this latter method. The experiment reported here was performed to investigate:

1. The influence of the application technique on the performance of the cleaning system;
2. The differences between physical and chemical sanitizing under field conditions; and
3. The advantages and disadvantages of field trials as a method of assessing the performance of cleaning systems.

2.6.1 EXPERIMENTAL DESIGN

This field experiment compared five systems. Four of the systems were those examined in the simulator in section 2.5 (see Table 2.5-1), namely: the acidified boiling water system (ABW), the triple system (T), the cold iodophor system (CI) and the cold alkali (CA). The fifth system, the warm iodophor system (WI) was identical to the warm iodophor system, used in the simulator experiments, except that the iodophor was applied at 50°C instead of at ambient temperature.

The five systems were applied to five groups of three farms. Over five treatment periods the systems were applied to all farms in a latin square design. Each treatment period lasted five weeks giving the whole trial a duration of twenty-five weeks.

The milking machines used ranged in size from 8 to 18 sets of cups (units) with one lowline and the remainder highlines. All farms supplied town milk year-round to the same treatment station. The

systems were applied to six of these machines by "reverse flow" (Twomey 1968b), a technique in which the cleaning solutions were forced at a high pressure through the machine in the opposite direction to that in which the milk normally flowed. The systems were applied by "circulation" on four farms - the solutions being passed once from a preparation trough through the machine in the same direction as the milk flow. The other five machines were "flush" cleaned, by drawing solutions through the machine from buckets in the same direction as the milk flow. All cleaning solutions were applied at a rate of 5 l/unit (set of cups).

2.6.2 FIELD TRIAL ASSESSMENT TECHNIQUES

GRAVIMETRIC ASSESSMENT OF SOIL

The rate of soil accumulation in the milking machines was determined by placing pre-weighed test pieces into the milk droppers (rubber tubes which convey the milk from the teat cups to the main milk line) for the five week treatment period, removing them and determining the weight changes. The test pieces were type 304 stainless steel tubes (125 mm long, 16 mm diameter) with a 2B finish. One tube was placed in each of four droppers on each milking machine for each treatment period.

Identification numbers were engraved on the outside of the tubes, which were then pre-cleaned by the method described in Section 2.1-4. The weighing of the tubes was performed by the technique described in Section 2.1-5. After pre-weighing, the tubes were inserted in the machine by cutting the rubber of the milk dropper, inserting the tube so the cut ends of the dropper met over the tube and taping over the join to prevent contamination of the exterior of the tube. After

removal from the machine the exterior of each tube was wiped with a tissue soaked in ethanol and then re-weighed as described in section 2.1-5.

BACTERIOLOGICAL ASSESSMENT

Swabbing Machine Surfaces to Obtain Contaminants

Each machine was sampled twice in each of the fourth and fifth weeks of each treatment period. At each sampling the machine was swabbed at ten sites: 2 teat cup liners, 2 milk droppers, 2 sight glasses, the end of the milk line, the receiving can, the milk cooler and the end of the milk delivery line. At each site an area of approximately 40 cm^2 was swabbed by a cotton swab moistened in a sanitizer-inactivator solution (MAF 1977).

Grading the Swab Contamination

The swabs were aseptically transported to the laboratory and within four hours steaked onto a TYGA plate and then a VRBA plate. The VRBA plates were incubated at 30°C for 24 h and the TYGA plates at 30°C for 48 h. The growth on the plates was then graded by the method described by the Ministry of Agriculture and Fisheries (1977):

Number of colonies	Grade
0	0,
1 - 70	1,
71 - 200	2,
201 - 650	3,
651 - confluent	4,
confluent growth	5.

The data are presented as "swab grades".

Classification of the Microflora of Machine Surfaces

At the final sampling of the fourth and fifth treatment periods, two sites were swabbed for the identification of contaminating microflora. One teat cup liner and one stainless steel milk dropper were swabbed as described above. At the laboratory the swab was placed in 10 ml of 0.1% peptone water and agitated. The rinse solution was then serially plated on 10^{-1}, 10^{-2}, and 10^{-3} decimal dilution plates of TYGA and incubated at $30^{\circ}C$ for 48h. Each dilution was plated in duplicate. After incubation up to 30 colonies were selected for identification from a plate containing 30 - 300 colonies. The selected colonies were identified by the method described in Section 2.1-8.

Bacteriological Quality of the Milk

The keeping quality of the milk from the refrigerated farm vat was assessed daily, using the Methylene blue, and Nitrate reductase tests (MAF 1977). Once each week coliform and thermoduric counts were also performed on the same samples (MAF 1977).

2.6-3 RESULTS

The mean soil weights, milk bacterial counts and swab grades of machine surfaces are presented for the five cleaning systems in Table 2.6-1. The data were examined by analysis of variance and the significances of the effect of any cleaning system are presented in the table. For any significant effect the means were examined by Duncan's new multiple range test and the significant differences are presented at the 0.05 level by the A, B, C system (Steel & Torrie 1960).

The mean soil weights, milk bacterial counts and swab grades of

Table 2.6-1: Mean deposit weights, milk bacterial counts and swab grades of machine surfaces with statistical analyses for 5 systems examined in field trial

Key:

x Significance of treatment differences by analysis of variance,

xx Standard error of treatment means,

xxx Significantly different means ($p < 0.05$) are denoted by a different letter,

xi Tryptone-yeast-glucose agar for a "total count",

xii Violet-red-bile agar for coliforms,

ABW Acidified-boiling-water system,

T Triple system,

CI Cold-iodophor system,

WS Warm-iodophor system,

CA Cold-alkali system.

Machine site	Parameter	Data	Cleaning system ABW	T	CI	WI	CA	* Sign. of Diffs	** SE of means
Whole machine	Deposit	Mean ($\mu g\ cm^{-2}$)	3.30	8.66	7.05	6.32	14.31	**	2.53
		Diffs xxx	C	B	BC	BC	A		
	Milk-coli form count	Log_{10} (mean + 1)/ml	2.01	1.74	2.25	2.31	2.38	+	0.24
		Diffs	AB	B	A	A	A		
	Milk-thermo-duric count	Log_{10} mean/ml	2.21	2.79	1.77	2.30	2.34	*	0.28
		Diffs	BC	A	C	AB	AB		
	Surface TYGA xi	Mean grade	1.37	1.61	1.44	1.36	2.08	***	0.15
		Diffs	B	B	B	B	A		
	Surface VRBA xii	Mean grade	0.39	0.50	0.53	0.50	1.05	**	0.16
		Diffs	B	B	B	B	A		
Teat cup liners	Surface TYGA	Mean grade	1.46	1.70	1.67	1.54	2.52	***	0.20
		Diffs	B	B	B	B	A		
	Surface VRBA	Mean grade	0.49	0.67	0.50	0.55	1.39	*	0.31
		Diffs	B	B	B	B	A		
Dropper tubes	Surface TYGA	Mean grade	1.21	1.35	1.19	1.15	1.56	NS	0.19
	Surface VRBA	Mean grade	0.34	0.34	0.24	0.27	0.67	*	0.13
		Diffs	B	B	B	B	A		
Sight glasses	Surface TYGA	Mean grade	1.86	1.74	1.76	1.44	2.33	NS	0.33
	Surface VRBA	Mean grade	0.72	0.62	0.53	0.66	1.12	NS	0.30
End milkline	Surface TYGA	Mean grade	1.66	2.23	1.90	1.90	2.87	**	0.34
		Diffs	B	AB	B	B	A		
	Surface VRBA	Mean grade	0.18	0.53	1.20	1.08	1.71	***	0.33
		Diffs	C	BC	AB	AB	A		
Rec. can	Surface TYGA	Mean grade	0.95	1.37	1.19	1.05	1.58	NS	0.26
	Surface VRBA	Mean grade	0.16	0.53	0.43	0.28	0.81	*	0.21
		Diffs	B	AB	AB	B	A		
Milk cooler	Surface TYGA	Mean grade	1.18	1.36	1.24	1.29	2.54	***	0.31
		Diffs	B	B	B	B	A		
	Surface VRBA	Mean grade	0.30	0.21	0.31	0.48	1.61	***	0.31
		Diffs	B	B	B	B	A		
Delivery tube	Surface TYGA	Mean grade	1.16	1.39	1.10	0.84	1.47	NS	0.29
	Surface VRBA	Mean grade	0.27	0.47	0.38	0.25	0.68	NS	0.25

machine surfaces are presented for the three application methods in Table 2.6-2. The results of the analysis of variance and Duncan's new multiple range tests ($p < 0.05$) are also presented.

The relative frequencies with which the groups of organisms occurred in the microflora on steel and rubber surfaces for four of the five cleaning systems are presented in Table 2.6-3.

2.6-4 DISCUSSION

Four of the five systems examined (ABW, triple, cold iodophor and warm iodophor) performed well (Table 2.6-1). The cold alkali system (CI) performed poorly in terms of soil weight, milk bacterial counts and swab grades of machine surfaces. The high TYGA grade for the whole machine resulted from inefficient cleaning of the teat cup liners, the end of milk line and the milk cooler. The high VRBA grade for the whole machine resulted from inefficient cleaning of the teat cup liners, the dropper tubes, the end of milk line, the receiving can and the milk cooler. It appears that the poor detergency of the system permitted the soil to accumulate to a level where the performance of the sanitizer was impaired. This may have resulted from a poor detergent formulation, or the limited solubility (1 g/l) of the detergent concentrate in cold water. This alkaline system supported a population consisting of bacilli, micrococci, streptococci and lactobacilli with a few flavobacteria, acinetobacter and other enterics.

Although the ABW system was the simplest, having only two components, it was also the most efficient in terms of detergency (Table 2.6-1). The lack of soil in those sections of the plant which are most difficult to clean, with the hot-water sanitizing, resulted in

Table 2.6-2: Mean deposit weights, milk bacterial counts and swab grades of machine surfaces, with statistical analyses, for 3 application methods in field trial

x Significance of treatment differences by analyses of variance,

xx Standard error of treatment means,

xxx Significantly different means ($p < 0.05$) are denoted by different letters,

xi Tryptone-yeast-glucose agar for "total count",

xii Violet-red-bile agar for coliforms.

Machine site	Parameter	Data	Application methods: Reverse flow	Circ-ulation	Flush	* Sign. of means	** SE of means
Whole machine	Deposit	Mean ($\mu g\ cm^{-2}$)	7.98	8.69	7.13	NS	1.66
	Milk-coliform count	Log_{10}(mean + 1)/ml	2.26	2.14	2.01	NS	0.20
	Milk-thermo-duric count	Log_{10} mean/ml	2.40	2.24	2.20	NS	0.21
	Surface TYGA xi	Mean grade Diffs xxx	1.93 A	1.29 B	1.48 B	*	0.20
	Surface VRBA xii	Mean grade Diffs	0.86 A	0.35 B	0.57 B	**	0.12
Teat cup liners	Surface TYGA	Mean grade	2.35	1.46	1.54	NS	0.49
	Surface VRBA	Mean grade Diffs	1.28 A	0.34 B	0.54 AB	+	0.37
Dropper tubes	Surface TYGA	Mean grade	1.45	1.16	1.27	NS	0.26
	Surface VRBA	Mean grade Diffs	0.58 A	0.21 B	0.33 B	*	0.12
Sight glasses	Surface TYGA	Mean grade Diffs	1.70 B	1.48 B	2.30 A	*	0.30
	Surface VRBA	Mean grade	0.73 A	0.39 B	1.07 A	*	0.21
End milk line	Surface TYGA	Mean grade	2.40	1.93	2.01	NS	0.49
	Surface VRBA	Mean grade	1.20	0.95	0.68	NS	0.34
Receiving can	Surface TYGA	Mean grade Diffs	1.79 A	0.86 B	1.03 B	*	0.34
	Surface VRBA	Mean grade Diffs	0.79 A	0.24 B	0.30 B	*	0.22
Milk cooler	Surface TYGA	Mean grade Diffs	2.38 A	1.23 B	0.96 B	***	0.22
	Surface VRBA	Mean grade	1.19	0.29	0.27	**	0.22
Delivery line	Surface TYGA	Mean grade Diffs	1.58 A	0.92 B	1.08 AB	*	0.28
	Surface VRBA	Mean grade	0.63	0.21	0.39	NS	0.20

the lowest swab grades for the teat cup liners, end of milkline and receiving can. The machine surfaces with the ABW system supported primarily thermoduric bacilli and micrococci, with some lactobacilli, flavobacteria, acinetobacter and coliforms (Table 2.6-3).

The triple system gave only satisfactory detergency and swab grades of machine surfaces (Table 2.6-1). The thermoduric count in the milk was the highest with the triple system, which may have resulted from the moderate detergency and the sanitizing rinse of hot water acting as a selective agent. This system also achieved the lowest coliform count.

The cold-iodophor system (CI) showed that a cold system can equal the hot systems in cleaning performance. Its detergency and machine surface grades were satisfactory (Table 2.6-1). Its coliform counts in the milk were slightly high, these organisms appearing to be concentrated in the distal end of the milkline. It gave the milk with the lowest thermoduric count showing that this cold system did not select the thermoduric organisms. The microflora consited primarily of coliforms and streptococci with far fewer bacilli and micrococci than the ABW system (Table 2.6-3). The only effect of increasing the temperature of the iodophor/acid wash solution from ambient to 50°C in the warm iodophor system (WI) was that the thermoduric count in the milk increased significantly and the microflora contained many yeasts (Table 2.6-3). The idea that the detergent temperature should exceed that of the melting point of butterfat (39°C) to assist in removal of fat was not supported in this trial.

There were no significant differences between the reverse-flow, circulation and flush application methods in terms of soil weights or milk bacterial counts (Table 2.6-2). However, in terms of the more

Table 2.6-3: Proportions (%) of different groups of organisms in the microflora on steel and rubber surfaces cleaned by 4 systems in the field trial

Organisms	Cleaning system															
	ABW				Cold Iodophor			Warm iodophor				Cold alkali				
	Steel	Rubber			Steel		Rubber	Steel	Rubber			Steel		Rubber		
	F1*	F1	F2	F3	F4	F5	F9	F8	F7	F8	F10	F13	F14	F15	F13	F15
Bacillus spp.	100	33	50	73	3		25		29		83	37	18	10	33	73
Micrococcaceae		17		8			25		14		17	57	65	47	48	
Lactobacillus spp.		33		4	17				14			3	6	3	4	
Streptococcus spp.						100			43			3	6	20	7	
Flavobacterium spp.		17		15									6	17	4	
Acinetobacter spp.			12		7			10		14						18
Coliforms			38		73		50	20		71						
Non-coliform enterics														3	4	9
Yeasts								70		14						
Total sample no.	6	6	8	26	30	4	4	30	7	7	6	30	17	30	27	11

* F1 Farm 1

sensitive bacterial swab grades of the machine surfaces some differences were observed. For the whole machine both circulation and flush were superior to reverse flow in terms of swab grade on TYGA, which resulted from those methods cleaning the receiving can, milk cooler and delivery line more efficiently than the reverse-flow. Reverse-flow also gave high VRBA grades for the whole machine which resulted from poor grades in the teat cup liners, dropper tubes, receiving can and milk cooler. Despite these differences all application methods were satisfactory.

SUMMARY

The performance of 5 cleaning systems was examined by field trial:

1. Two systems incorporated sanitizing by hot water. The system with the acidic washing solution was the best of the five systems, while that with the alkaline detergent was only moderately effective;
2. Two systems used cold solutions and chemical sanitizing. One of these (cold iodophor) performed very well, while the other (cold alkali) was unsatisfactory;
3. The elevation of the temperature of the iodophor washing solution from ambient to 50°C did not improve the performance of the system;
4. The microflora of the surfaces of the machines reflected whether heat was applied, the chemical nature of cleaning solutions used and the amount of soil on the surfaces.

3

GENERAL DISCUSSION

3.1 PERFORMANCE OF THE CLEANING SYSTEM

The most important measures of the performance of a cleaning system are the control of both soil accumulation and the development of micro-organisms on surfaces of the equipment. There are many secondary aspects of system performance including; damage of the surfaces of equipment, contamination of the food by residues of the cleaning solutions, and solution effluents polluting the environment. In this study soil accumulation and bacteriological control have been the parameters assessed.

3.1.1 CONTROL OF SOIL ACCUMULATION BY THE CLEANING SYSTEM

In the experiments described previously the amount of soil increased on all surfaces with increasing cycle number. The nature of the soil deposition curves varied greatly, with linear, accelerating or diminishing rates and plateau-shaped relationships (e.g. Figure 2.5-1). The many factors which interact to influence soil accumulation are discussed below.

THE SUBSTRATE

The surface characteristics of the substrate and the material of which it is made are extremely important as they influence the

nature of the soil which accumulates. The surface characteristics which affect the soil include; smoothness, surface finish, porosity, hardness, wettability by a liquid soil component, geometry and chemical reactivity with the soil (Jennings 1965). The nature of the surface influences the rate of soil deposition, but has little effect upon the rate of soil removal (Pflug et al.1961). With continuing use a surface may become physically abraded or corroded, which damages the smooth surface and renders it unhygienic (Clegg 1962).

The soil was found to accumulate on glass and stainless steel surfaces to a similar degree (Figures 2.5-1a, 2.5-1c), but had different accumulation characteristics. The soil accumulated linearly on steel with Systems D.2, D.3 and D.4, while on glass the soil changed mass in early cycles, but remained unchanged thereafter. Glass is an admirable surface for dairy equipment because of its hardness, smooth surface, corrosion-resistance and transparency. Glass surfaces have excellent cleanability which results from their greater resistance to soiling, and the more efficient removal of the soil that is deposited (Moore et al. 1951; Pflug et al. 1961; Ridenour & Armbruster 1953). Stainless steel has similar cleanability to glass, but has further advantages in that it is much easier to fabricate and resists impact damage. However, it lacks the transparency and corrosion resistance of glass (Clegg 1962).

Rubber and rubber-like materials are used where flexibility is required (such as teat cup liners, tubing and gaskets). Rubbers often have a smooth surface initially, but this can be mechanically or chemically abraded, and they can also lose their elastic properties and dimensional stability. This surface deterioration is

demonstrated in Figure 2.5-7a. A soil of fat and protein accumulated on the surfaces (Kiermeier et al. 1968). The amount of soil which accumulated was approximately ten times greater than that on glass or steel. The surface cracks which eventually occur provide bacteria with sites protected from cleaning agents, and when this is combined with the greater soil mass, bacteriological control of rubber surfaces is difficult to achieve (e.g. Major 1962b).

THE SOIL

The nature of the soil greatly influences the ability of the detergent to remove it. The soil characteristics which influence the soiling process include particle size, viscosity, surface tension, mutual solubility of soil components and their chemical reactivity with the substrate (Jennings 1965). Cold milk is a stable emulsion, the stability of which can be disturbed by drying, rinsing, detergents and sanitizers. The composition of the soil which results varies greatly with the surface, cleaning system and site within the milking machine, but has a typical composition of; water - 5%, fat - 1%, protein - 60% and minerals - 32% (Twomey 1975).

THE DETERGENT

The efficiency of a detergent within the system is dependent upon the characteristics of the detergent itself and also the other components of the system. The class of detergent (heavy-duty alkaline, general purpose or acidic) defines the general characteristics of a detergent and variations in the formula within a class greatly influence its cleaning performance. The gross effect of detergent reagents is shown by the great difference in performance between Systems A.4 and A.2 in Figure 2.2-1, where the only differ-

ence was the addition of detergent reagents to the "washing" solution .

The concentration of the detergent is a most important factor in determining its performance. With those detergents which have a surface-active effect, or function by adsorption and/or displacement, detergency will improve with increasing concentration to a saturation maximum (Harris & Satanek 1961a, 1961b; Jones 1961; Kling & Lange 1955). The performance of materials such as strong acids or alkalis, which do not function in that way, increases with increasing concentration with no maximum (Jennings 1959).

The affect of temperature on detergency is complex. On fatty soils there is a small linear increase in cleaning performance with increasing temperature (Bacon & Smith 1948). On more complex soils, particularly those containing protein, there is an optimum detergent temperature (e.g. 65°C for milk soil - Schlussler 1970). Also, surface-active detergents display an optimum temperature, while simpler detergents like sodium hydroxide increase efficiency linearly with temperature (Jennings 1959, 1965).

The contact-time between detergent and soil also influences the detergent performance in a manner described by the relationship (Bacon & Smith 1948):

$$\text{Soil removal } (S) = K\,(CFT)^n$$

Where K = a constant,

C = concentration of detergent,

F = mechanical force of cleaning,

T = contact time, and

n = slope of linear portion of $\frac{d \log S}{d \log T}$

In milking machine cleaning the contact-time can be increased by; increasing the volume of detergent put through a machine that is flush - cleaned, recirculation, immersion of the machine components in detergent (bucket plants) or leaving the detergent in the lines of pipeline machines. The contact-time may extend from a few minutes to the complete intercycle period.

The relationship of Bacon & Smith (1948) detailed above shows the importance of solution turbulence in detergency. There are three phases in the cleaning of a milk soil; a mechanical effect below which no increase in detergency over an unagitated solution is achieved, a phase in which detergency increases with mechanical effort, and a mechanical effort over which no further increase in cleaning efficiency is achieved (Schlussler 1970). In the non-turbulent soak conditions applied in the simulator experiments emphasis was placed on the physico-chemical activity of the detergent. When soak systems are used in milking machines in practice it is desirable that some turbulence be applied, preferably after the soak period, and possibly also at the start of that period. The importance of turbulence is demonstrated in the differences in the relative amounts of soil deposited in the simulator (Figure 2.4-1a), and field experiment for the same systems (Table 2.6-1). For example, the triple system had a soil mass of 63 $\mu g/cm^2$ after 36 cycles in the simulator, but only 8.7 $\mu g/cm^2$ after 70 cycles (35 days) in the milking machine.

WHOLE SYSTEM EFFECTS

The detergent was the single most effective component of the system in controlling soil accumulation (cf Systems A.2 and A.4 in

Figure 2.2-1). The efficiency of the detergent was improved by a pre-detergent rinse which removed some of the loosely adhered soil and reduced the soil load to which the detergent was exposed. The addition of a post-detergent rinse also reduced the deposit slightly by assisting the removal of any detergent-soil complexes which were not drained away after the detergent.

The detergency of the total system was improved by the addition of an iodophor sanitizer (Figure 2.3-1), although the effect of the iodophor differed with its timing within the system. The deposit was reduced markedly with the systems in which the iodophor was applied for a short period only, whether as a post-wash or pre-milk solution.

The addition of an acidic detergent to the system in the complementary cleaning experiment increased the soil deposition on both steel and rubber surfaces (Figure 2.4-1). A similar effect was observed with the acidified-boiling-water system on steel, rubber and glass surfaces (Figure 2.5-1), although this system gave the lowest deposit in the practical milking machine situation (Table 2.6-2). It is possible that the acidic solutions cause milk to be deposited as a friable protein soil (e.g. Figure 2.5-3a), which was not removed in the soak conditions existing in the simulator, but which was broken up by the solution turbulence within the pipeline milking machine.

PERIODIC CLEANING

When the soil on equipment surfaces accumulates to an unsatisfactory level an alternative cleaning regimen is applied which will remove the deposit. This periodic cleaning regimen may use the normal cleaning solutions at increased concentration and/or temperat-

ures, or may use completely different, and usually more active materials. The acidic detergent applied periodically in the complementary cleaning experiment was found to be unsatisfactory for this task as it lacked the necessary detergency (Figure 2.4-1a - System C.1).

3.1.2: BACTERIAL CONTROL BY THE CLEANING SYSTEM

PERFORMANCE OF THE TOTAL SYSTEM

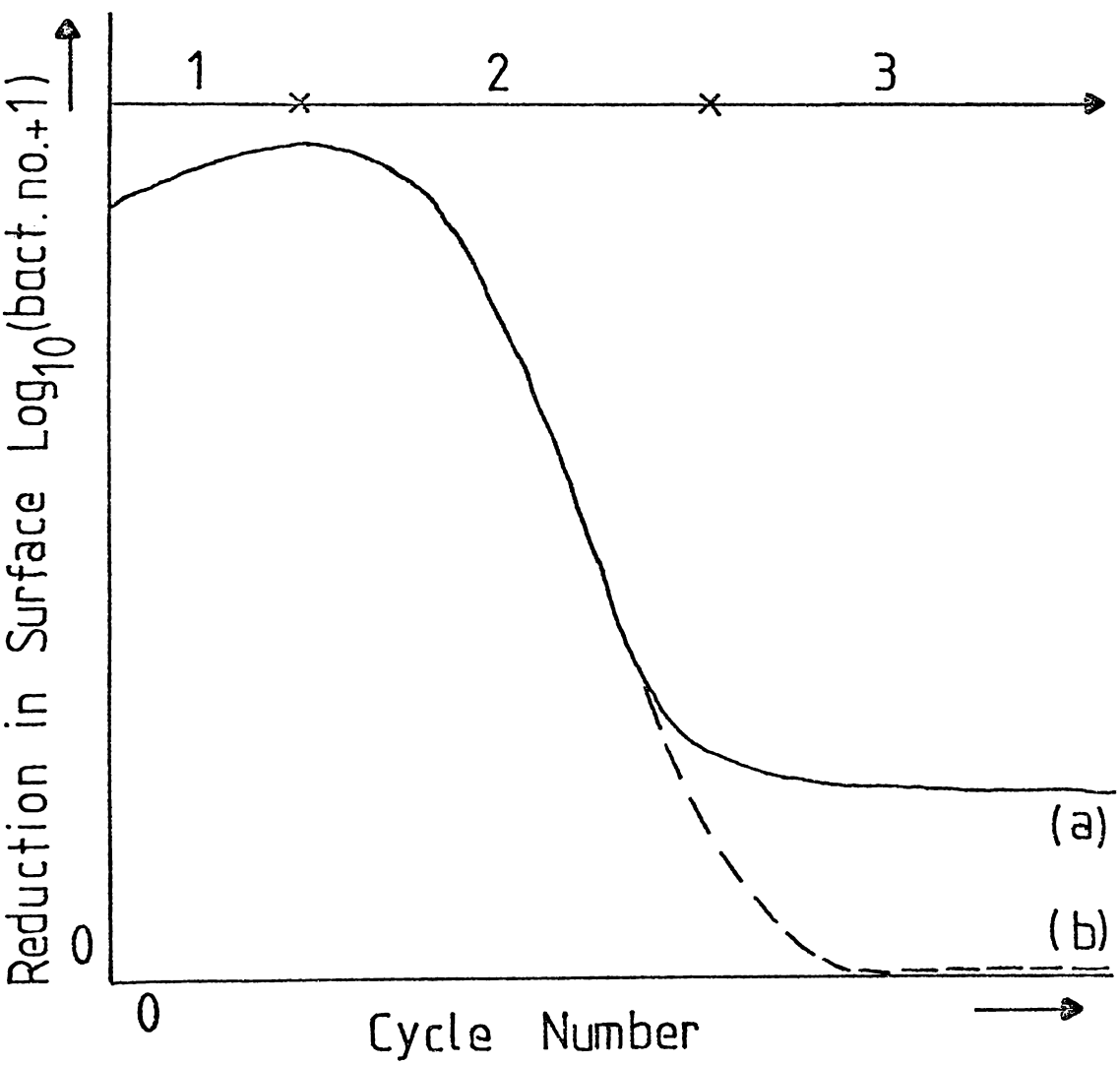
The performance of a system measured in terms of the reduction in the bacterial count is the sum of the effects of the individual components. It is the resultant of the increase in numbers from soiling, contamination and intercycle growth, and the decrease in numbers from the removal of bacteria or kill of bacteria by cleaning solutions, together with death occurring during the intercycle period. The effect of any individual component of the cleaning system diminished as the amount of soil increased over multiple cycles; this resulted in the diminishing performance of the total system (e.g. Figure 2.2-2a).

Change in System Performance with Time

The bacteriological performance of a cleaning system can be measured in terms of the reduction in count on the equipment surfaces from immediately after soiling to immediately before the next soiling. A general curve for the change in this performance with time is presented in Figure 3.1-1. This curve has three phases:

1. Increasing-effect phase; in this phase the soil level is insufficient to protect the organisms from the system and the system eliminates all viable organisms. There is an increase in the numbers of organisms removed, because the number attached to the surface after soiling (i.e. the numbers available for treatment by the system increases

Figure 3.1-1: Change in the bacteriological performance of a cleaning system with time


1
2
3
Reduction in Surface Log10(bact. no.+1)
0
0
Cycle Number
(a)
(b)

with time and the organisms there are completely eliminated.

2. Diminishing effect phase; in this phase the soil level has reached a level where partial protection from the cleaning system is afforded the organism. The system performance diminishes as the amount of protection increases with further soil deposition.
3. Constant effect phase; in this phase the cleaning system has reached equilibrium with the soiling system and the count reduction is constant. There are two types of constant phase - a true equilibrium (a) or a situation where the system has no effect at all (b).

In the experiments reported here no single system displayed all three of the phases described. However, the increasing-effect phase is observable in System B.3 (post-wash sanitizer), System B.4 (inter-cycle sanitizer - Figure 2.3-12c, -12d), System C.3 (all acid) and System C.4 (iodophor - Figure 2.4-11b,-11d). The diminishing-effect phase was observed with most systems (e.g. Systems B.1 and B.2 in Figure 2.3-12c). Most of the experiments reported ran for too short a period for the systems to reach the constant-effect phase, but a true equilibrium was established with System A.2 (no final rinse). In later cycles System A.4 (no detergent) had no effect on the bacterial count at all. The more efficient systems were those which perform in the increasing or diminishing effect phases for longest time. In very inefficient systems the increasing effect phase was so short as to be indeterminable under the experimental conditions.

Effect of Soil on System Performance

The effect of any cleaning solution, or total cleaning system,

upon bacteria on the equipment surface will be influenced by the nature and amount of the soil on the surface, and the relationship between the organism and the surface/soil environment. It is possible that on a clean surface a solution may achieve absolute bacterial control, leaving no survivors. As the amount of soil on the surface increases (over multiple cycles) a stage may be reached where there is sufficient soil to protect some organisms and permit them to survive. This is depicted graphically in Figure 3.1-2. A further increase in the soil level may permit the survival of all of the organisms - the solution having no effect.

The minimum soil mass at which the first organisms survive the treatment will hereafter be referred to as the Total Effect Maximum - TEM (i.e. the maximum soil level at which no organisms survive) The lowest soil mass at which there is no reduction in the numbers of surface organisms is defined as the Zero Effect Minimum - ZEM. The range of soil mass between the TEM and ZEM is the zone in which the component or system will be partially effective.

This type of analysis has been applied to all of the systems from the simulator experiments on depletion (2.2) sanitizer timing (2.3) and complementary cleaning (2.4). The data presented in Table 3.1-1 show that only two of the systems (A.3 and B.1) reached zero effect minima within the experimental periods. However, a number of the systems did not achieve total bacterial control (A.3, B.1, B.3 and C.2). These levels reflect the total performance of the system, rather than one specific feature.

Figure 3.1-2: Assessment of the influence of soil mass on the performance of cleaning systems and system components

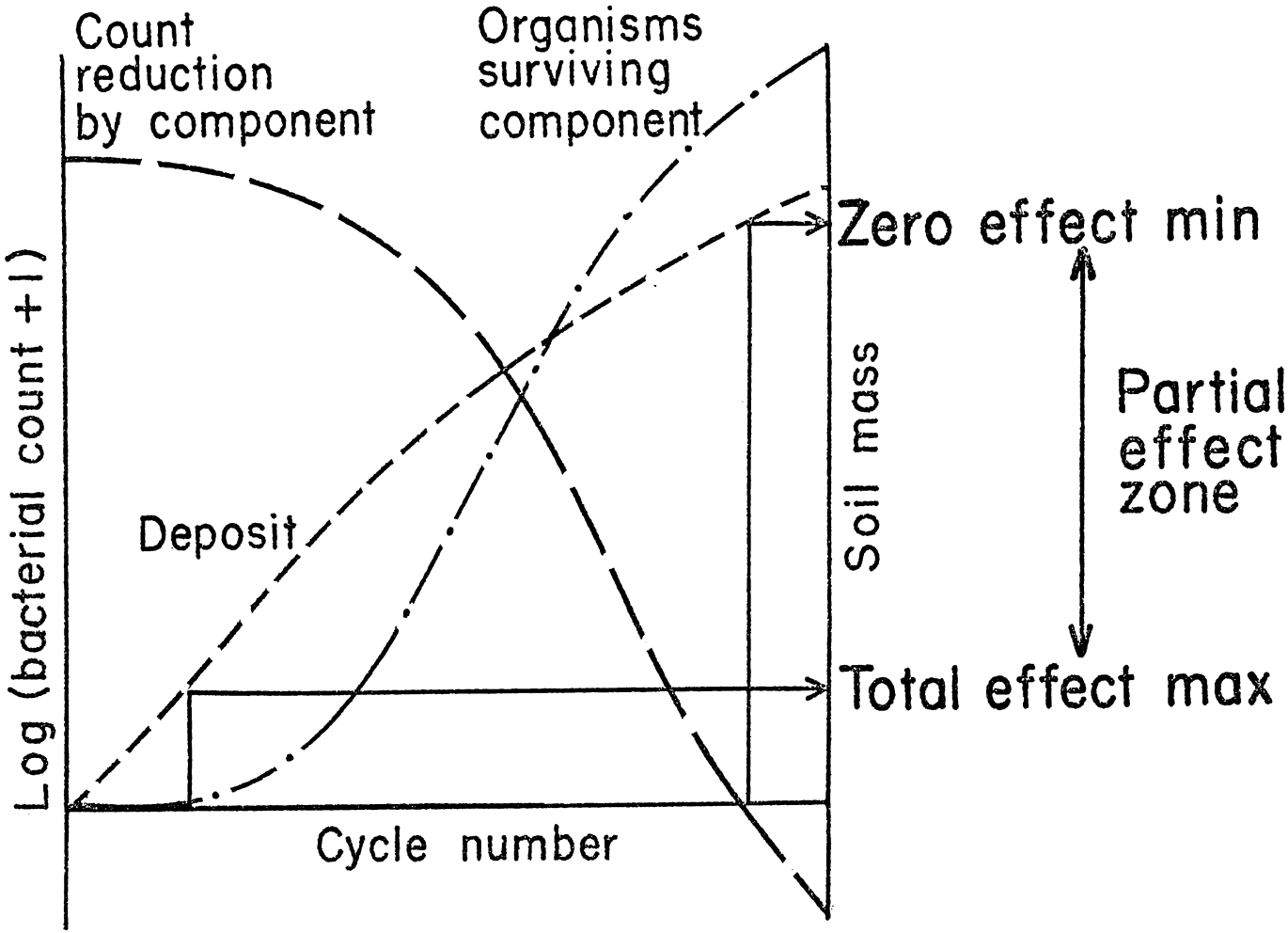

Count
reduction
by component
Organisms
surviving
component
Zero effect min
Partial
effect
zone
Soil mass
Deposit
Total effect max
Log (bacterial count + 1)
Cycle number

Table 3.1-1: Critical soil levels for 12 cleaning systems studied in 3 experiments

Cleaning system	Section of experiment	Organism	Critical soil levels ($\mu g/cm^2$)		Source of data
			Total effect maximum	Zero effect minimum	
A.1 Triple	2.2	S. faecalis	5	> 20	Figures 2.2-9a, -9b
A.2 No post-wash rinse	2.2	S. faecalis	10	> 78	
A.3 No post-milk rinse	2.2	S. faecalis	-	20	
A.4 No detergent	2.2	S. faecalis	45	> 440	
B.1 No sanitizer	2.3	S. faecalis	-	60 est.	Figures 2.3-12a,-12d
		E. aerogenes	-	58 est.	
B.2 Pre-milk sanitizer	2.3	S. faecalis	-	> 20	
		E. aerogenes	8	> 20	
B.3 Post-wash sanitizer	2.3	S. faecalis	-	> 40	
		E. aerogenes	-	> 40	
B.4 Intercycle sanitizer	2.3	S. faecalis	> 75	$\gg$ 75	
		E. aerogenes	> 75	$\gg$ 75	
C.1 Periodical acid	2.4	S. faecalis	> 38	$\gg$ 38	Figures 2.4-11a,-11d
		E. coli	> 38	$\gg$ 38	
C.2 No acid	2.4	S. faecalis	-	> 50	
		E. coli	25	> 50	
C.3 All acid	2.4	S. faecalis	> 300	$\gg$ 300	
		E. coli	> 300	$\gg$ 300	
C.4 Iodophor	2.4	S. faecalis	> 125	$\gg$ 125	
		E. coli	> 125	$\gg$ 125	

PERFORMANCE OF COMPONENTS OF THE SYSTEM

Detergents

White (1962) stated that "bacteria are dirt. In theory it should be possible to 'sanitize' a surface using non-sanitizing detergents." Indeed, the fact that very high proportions (up to 99.8%) of both the soil and bacteria have been shown to be removed by simple cleaning has demonstrated that detergency is quantitatively more important than sanitizing (Hays et al. 1968; Mallmann et al. 1953; Mitchell & Clifford 1964). The important contribution of the detergent to the performance of the total system has been demonstrated in this work also (cf Figures 2.2-3b and 2.2-5b, where the only difference is the addition of detergent reagents to the washing solution).

The performance of the detergent is greatly influenced by the amount of soil on the substrate. The inverse relationship between detergent performance and amount of soil was demonstrated in both the relative effectiveness of the detergent between systems and the reduction in detergent efficiency with increasing cycle number (Figure 2.3-9).

Hays et al. (1968) found that an alkaline detergent did not completely eliminate the live bacteria. This is demonstrated by the absence of a total effect maximum with this system component (i.e. there were always some survivors - (Table 3.1-2). This was not the case with the acidic solution which was far more efficient than the alkaline detergent, having significant total effect maxima, and not reaching any zero effect minima within the duration of the experiment.

The extremely efficient bacterial control achieved by the acidic detergent in System C.3 (all acid - Figure 2.4-4a - final solution)

Table 3.1-2: Critical soil levels for components of cleaning systems in 3 simulator experiments

Cleaning system component						Organism	Critical soil levels ($\mu g/cm^2$)		Source of data
Class of component	Solution	Site in system	Section of experiment	System			Total effect maximum	Zero effect minimum	
Rinse	Water, 200 mg/l total hardness (as $CaCO_3$), 20°C	Pre-milk	2.3	B.1	(no sanitizer)	S. faecalis	-	0	Figure 2.3-10
						E. aerogenes	-	0	
				B.3	(post-wash sanitizer)	S. faecalis	-	0	
						E. aerogenes	-	0	
		Post-milk	2.2	A.1	(triple)	S. faecalis	-	0	Figures 2.2-6c,-6d
				A.2	(no post-wash rinse)	S. faecalis	-	0	
				A.4	(no detergent)	S. faecalis	-	0	
		Post-detergent	2.2	A.1	(triple)	S. faecalis	-	0	Figures 2.2-7c,-7d
				A.3	(no post-milk rinse)	S. faecalis	-	0	
Detergent	Alkali, 5 g/l, 50°C	After post-milk rinse	2.2	A.1	(triple)	S. faecalis	-	$\gg$ 37	Figures 2.2-7a,-7b
				A.2	(no post-wash rinse)	S. faecalis	-	$\gg$ 80	
		After milk	2.2	A.3	(no post-milk rinse)	S. faecalis	-	25	
	Acidic, 2 ml/l, 20°C	Final soln - constant	2.4	C.3	(all acid)	S. faecalis	0	$\gg$ 150	Figure 2.4-9
						E. coli	> 150	$\gg$ 150	
		- periodic	2.4	C.1	(periodic acid)	S. faecalis	> 45	$\gg$ 45	
						E. coli	> 45	$\gg$ 45	
Sanitizer	Iodophor	Pre-milk, 25 mg Av. I/l	2.2	A.1	(triple)	S. faecalis	5	18	Figures 2.2-6c,-8d
				A.2	(no post-wash rinse)	S. faecalis	10	$\gg$ 80	
				A.3	(no post-milk rinse)	S. faecalis	-	25 est.	
				A.4	(no detergent)	S. faecalis	45	150	
			2.3	B.2	(pre-milk sanitizer)	S. faecalis	-	10	Figure 2.3-11
						E. aerogenes	-	> 20	
		Post-wash, 25 mg Av. I/l	2.3	B.3	(post-wash sanitizer)	S. faecalis	-	42	
						E. aerogenes	n.a.*	> 42	
		- 75 mg Av. I/l	2.4	C.4	(iodophor)	S. faecalis	17	$\gg$ 300	Figure 2.4-9
						E. coli	> 300	$\gg$ 300	
		Intercycle, 25 mg Av. I/l	2.3	B.4	(intercycle sanitizer)	S. faecalis	> 75	$\gg$ 75	Figure 2.3-11
						E. aerogenes	> 75	$\gg$ 75	

* n.a. not available

demonstrated the advantage of a combined detergent-sanitizer, over a detergent with very weak bactericidal activity. Any bacteria removed from the surface would be killed in solution by a detergent-sanitizer, preventing the redeposition of live bacteria which may occur with a detergent (Davis 1968). Combined detergent - sanitizers also offer advantages in terms of the costs of cleaning. The disadvantages of the combined product are that the sanitizer component of the detergent-sanitizer has to operate in sub-optimal conditions of pH, and formulation ingredients, and in a medium which contains a relatively high amount of milk soil. This latter disadvantage can be minimised by application of an effective pre-detergent (post-milk) rinse. This is shown by the lower ZEM value of the post-milk detergent, where the high soil load reduced detergent performance (Table 3.1-2).

Sanitizers

The iodophor sanitizers contributed significantly to the control of soil accumulation (see Systems B.2, B.3 and B.4 in Figure 2.3-1). Thus the iodophor functioned by both removing and killing the bacteria.

The contribution of the iodophor sanitizer to the performance of the total system ranged from insignificant, to being the most effective single component. In this work the ability of the sanitizer to reduce the numbers of viable organisms on surfaces has been shown to be controlled by a number of factors:

1. The concentration of the sanitizer greatly influences performance (Clegg 1962). Increasing the concentration of the iodophor from 25 to 75 mg Av. I/l and the phosphoric acid concentration from 0.24 to 1.24 ml/l improved the performance of this sanitizer, expressed in both higher TEM and ZEM values (Table 3.1-2).

2. The contact time can also be important in determining sanitizer performance (Clegg 1962). The long contact-time of the inter-cycle sanitizer (System B.4) made it far more efficient than either of the short contact-time methods (Systems B.2 and B.3 in Figures 2.3-11b,-11d) despite having a greater amount of soil on the surface (Figure 2.3-1). Maxcy and Shahani (1960) achieved satisfactory bacteriological control by placing a low-concentration sanitizer solution into equipment for the whole of the inter-cycle period.
3. The surface being sanitized greatly influences the performance of a sanitizer - old rubber or plastic surfaces, or corroded metals limit the bacterial control achieved (e.g. Clegg 1962; Daufin 1973; Major 1962b; Maxcy et al. 1971).
4. The soil on a surface may reduce the efficiency of a sanitizer (Clegg 1962). The pre-milk iodophor sanitizer in systems A.1 - A.4 were totally effective in bacterial control up to a soil mass (TEM) of from 5 to 65 $\mu g/cm^2$ (Table 3.1-2). As the soil mass increased it reached a level where the bacteria were totally protected from the sanitizer (ZEM) at soil masses ranging from 18 to 150 $\mu g/cm^2$. Similar information is available for all of the other iodophor applications studied in the simulator experiments.
5. The timing of sanitizing within the cleaning system has been studied by field trial on milking machines. Middleton et al. (1965) showed that, a chlorine sanitizer was more effective applied before milking rather than post-washing. In the simulator experiment performed in this study the application of the iodophor was slightly more efficient in reducing the bacterial count when applied as a post-wash solution, than when applied immediately

before milking (Figures 2.3-11a,-11c). This may have resulted from the bacteria being more susceptible to stresses (alkaline detergent and iodophor) applied sequentially, than when separated by a 9.5 h recovery period. Also, the residual sanitizer chemicals which remained on the surfaces after the drainage of the post-wash sanitizer. reduced the bacterial numbers still further during the intercycle period (Figure 2.3-9b), which resulted in the total system being more effective (Figure 2.3-12c). Thus, the sanitizer may be applied after milking, as long as measures are taken to prevent recontamination of the surface between sanitizing and the next milking. The difference in favour of the pre-milking sanitizer in the field (Middleton et al. 1965) may reflect the ability of that application to prevent recontamination, whereas in the controlled conditions of the simulator, this recontamination could not occur, favouring the post-wash application.

6. Periodic treatment with heat to reduce the surface flora, or detergents to remove accumulated soil both improve the efficiency of subsequent sanitizing (Clegg 1962; Davis 1968; Whittlestone & Phillips 1959). The effect of a periodic treatment which had a bactericidal, but not detergent, action is shown by System C.1 in Figure 2.4-11b or-11d. Here the efficiency of the system increased markedly for the cycle that the treatment was applied, but the system performance in non-periodic treatment cycles was unaffected. If a periodic treatment was applied which was an efficient detergent and sanitizer the surface condition would be returned to the starting point and the characteristic change of performance with time would follow.

7. The performance of a sanitizer is partly determined by the efficiency of the other components of the system. An identical iodophor sanitizer was applied before milking in all four systems in the experiment on depletion effects, but the efficiency of the sanitizer varied as the other components of the system were altered (Figure 2.2-8d).

Other factors which influence the performance of the sanitizer, but were not examined in these experiments include:

1. The type and formulation of the sanitizer, which affect its speed of action, penetration of soil, wetting of bacteria, specifity and persistence on surfaces (Clegg 1962; Leggat 1969); and
2. The application method (flow, spray, aerosol, immersion (Rice 1962).

Rinses

In the soak conditions which prevailed in the simulator, the water rinses were very inefficient system components, having no effect on the surface bacterial numbers, the zero effect minimum occurring at zero soil mass. The timing of the rinses in the cleaning system did not alter their performance, as both pre- and post-milk rinses performed similarly to post-detergent rinse (Table 3.1-2). They did improve the ability of the detergent to remove soil and therefore limit the numbers of organisms that the surfaces were able to support. In most practical food industry situations, the post-product rinse is applied as a flowing solution, which will not only remove non-adsorbed soil, but also some adsorbed soil and attached organisms.

Contaminated rinse waters can have a very significant deterimental effect on system performance, particularly when applied immed-

iately before the product (Figures 2.3-2b and 2.3-4b).

Soil Depth

The soil masses are sometimes of more value when examined as soil depths. If the density of the soil is considered to be 1 g/cm^3, a soil mass of Z $\mu g/cm^2$ is equivalent to a mean soil depth of Z x $10^{-2}\mu m$. Thus the Total Effect Maximum of 5 $\mu g/cm^2$ for the pre-milk iodophor in the triple system (Table 3.1-2) is equivalent to a mean soil depth of 0.05 μm or approximately one-twentieth the diameter of a coccoid organism. It is unlikely that this amount of soil is sufficient to inactivate the sanitizer, nor is it sufficient to protect the organism physically. This suggests that the soil must be unevenly distributed and surviving organisms are associated with the deeper regions of the soil (see Figure 2.5-5a). Similarly, the Total Effect Minimum of 18 $\mu g/cm^2$ for the same organism is equivalent to a mean soil depth of 0.18 μm, which is insufficient to submerge an organism, and protect it from the sanitizer, again indicating uneven soil distribution. The soil depths at which Total Effect Maxima and Zero Effect Minima occur require further study under more precisely controlled conditions, if they are to be accurately determined.

3.1.3 IMPLICATIONS OF THE DATA

The data on the performance of the cleaning system have a number of theoretical and practical implications.

1. Current attempts to simplify cleaning systems by reducing the numbers of components can be facilitated by three practices. The first of these is to replace detergents by combined detergent-sanitizers. The inability of the alkaline detergent to eliminate bacteria (Figure 2.2-7a) compared to the acidic and iodophor

detergent-sanitizers (Figure 2.4-9a, 9c) demonstrated the advantages of the combined type of product. In this situation the increased cost of using a slightly higher concentration of sanitizer to permit satisfactory bactericidal activity in high soil loads is more than offset by the costs of labour saved.

The second method is to minimise the number of rinses. A post-detergent rinse is usually recommended to remove any residual detergent and soil. However, if the detergent is freely draining, this post-detergent rinse can be eliminated without deleterious effect. In fact, the residual detergent (or detergent-sanitizer) chemicals remaining on the surfaces during the inter-cycle period will assist in bacterial control. The possibility of eliminating the post-milk rinse should be examined in practical conditions.

Although replacement of a sanitizer applied as a pre-milking rinse by a post-wash application does not reduce the number of components in the system, it does make cleaning easier for the dairymen, which can pay dividends in the reliability of his performance. Under laboratory conditions this practice was found to be slightly superior in bacterial control to the pre-milking practice, but in the practicalsituation steps should be taken to prevent recontamination of the machine prior to the next milking.

2. A soak cleaning system comprised of cold solutions must perform satisfactorily without the dual advantages of heated solutions - improved detergency and bactericidal activity. The soak application eliminates the great benefit of solution turbulence. These problems can be overcome to some degree by the following type of system:

1. Post-milking water rinse - applied turbulently; followed by
2. Wash with detergent-sanitizer - by immersion for complete intercycle period - removed from surface turbulently (brushing or turbulent fluid flow).

This has the advantage of the turbulent rinse removing the majority of the milk soil and reducing the soil load on the detergent. The efficiency of this rinse may be improved by inclusion of a rinse additive (Twomey 1966a). The detergent-sanitizer has a very long contact-time for action , which also prevents any growth in the intercycle period. The turbulent removal of the detergent-sanitizer is essential if efficient detergency is to be achieved. The detergent-sanitizer used should be alkaline as acidic solutions (either hot or cold) were shown to be unsatisfactory in the simulator where turbulence was limited.

3. The Total Effect Maxima (TEM) and Zero Effect Minimum (ZEM) provide useful performance parameters for systems or components of systems. If a cleaning system could be applied in such a way that its TEM was never exceeded, there would be no bacteria surviving the system and there could be no contamination of the milk at all. This is not usually achieved in practice and the system is operated in the partial effect zone (Figure 3.1-1). As the soil level increases in this zone the contamination from the surface into the milk will reach a level where it becomes excessive. At this point periodic cleaning must be performed in order to lower the mass of soil on the surfaces to an acceptable level. Thus, the soil mass on the surface will start cycling between the low level and the level at which the periodic cleaning is applied.

4. The difficulty of cleaning rubber surfaces and plastics (e.g. Wildbrett 1973b) means that components of the milking machine manufactured from these materials become the major sites of bacterial growth. Consequently the use of these materials must be minimised until either better formulations are available, or detergents and sanitizer more capable of cleaning them are developed.

3.1.4 FURTHER WORK REQUIRED

The work reported has answered some of the questions asked, but demonstrated the need for work in several important areas.

1. The contribution of each component to the performance of the total system, in terms of both deposit and bacterial control, should be studied in the industry situation. The results of the simulator study should be tested under practical conditions.
2. The cold iodophor system was shown to be a satisfactory system in all but control of Gram-negative organisms. This system should be developed, to overcome this problem. This may require different approaches to;
   a. the pipeline machine - where the turbulence permits use of acidic cleaning solutions,
   b. soak-cleaned machines - where the lack of turbulence indicates a preference for alkaline detergents.

   The use of soak cleaning creates an opportunity for re-use of the detergent-sanitizer. A careful examination of the detergency and sanitizing limits to re-use should be made.
3. The relative contributions of removing and killing of bacteria as control mechanisms should be made under a range of practical

conditions.

4. The need for and practice of periodic cleaning with complementary solutions should be examined from the viewpoints of both detergency and sanitizing.

3.2 THE CONTAMINATION CYCLE BY BACTERIA ON SURFACES OF MILKING MACHINES

A primary function of the cleaning system applied to milking machines is that of limiting the bacterial contamination of the milk to an acceptable level. This is achieved by controlling the numbers of organisms removed from the equipment surfaces into the milk. This contamination process is the final event in a cycle which may be summarised as follows:

1. Deposition of bacteria onto the equipment surface,
2. Attachment of bacteria to the equipment surface,
3. Depletion of bacterial numbers by components of the cleaning system,
4. Development during the intercycle period; and
5. Contamination of the milk the next time it passes over the surface of the equipment.

As these events may occur at every use of the milking machine, a "contamination cycle" is established. This contamination cycle had not been studied as a whole, although some aspects of it have been examined in isolation (e.g. the depletion of bacterial numbers by sanitizers). The experiments reported in this work have provided some information on the nature of the contamination cycle.

3.2.1 COMPONENTS OF THE CONTAMINATION CYCLE

DEPOSITION OF BACTERIA ONTO EQUIPMENT SURFACES

As discussed in Section 1.5.1, there are six sources of organisms which deposit onto the surfaces of milking machines. These are the milk, air, cleaning solutions, personnel, insects and cleaning

equipment. Of these only the milk and cleaning solutions are quantitatively of any significance (Benham & Egdell 1970; Cousins 1972; Neave et al. 1962). The pasteurised milk used for soiling in the simulator experiments contained a small population of thermoduric organisms ($\sim 10^3$/ml) and a large number of dosing organisms ($\sim 10^6$/ml). Some organisms of each of these groups would have been deposited on, or close to, the surface with the soiling milk. Studies of the microflora of the surfaces in subsequent cycles show that some of the deposited bacteria became attached to the surface (Tables 2.4-2 and 2.5-3).

Quality control counts which were routinely performed on the cleaning solutions used in the simulator experiments showed than none of the solutions which contained detergents, sanitizers or rinse additives had any bacterial contaminants. However, the following water rinses contained bacteria; the pre-milk rinse of Systems D.1 and D.3, the final solutions of System D.2 and post-detergent rinses in all systems of the experiment on the timing of sanitizing. The bacteria present in these rinses grew on VRBA and would consequently have been included in the surface counts of E. aerogenes. This contamination was very important in these systems where the water was used as a pre-milk rinse (D.1 and D.3) as the surface count was increased by up to 2 $\log_{10}$ units, immediately prior to soiling by the milk (Figure 2.3-10d). Thus, significant contamination of the milk would have occurred. Where the rinse was used as the final solution in System D.2 it raised the $\log_{10}$ surface count by 0.8. This ultimately increased the numbers which remained after the pre-milk sanitizer and effected contamination of the milk (Figure 2.3-3b). This type of rinse-water contamination is important, not only because

of the numbers of organisms thus contributed, but also for the variety of types of organisms introduced. This variety increases the chance of effective surface-colonizers being included in the organisms deposited on the surfaces.

As well as being the major sources of depositing organisms, both milk and cleaning solutions are significant vectors of organisms within the milking machine. The major concentration of contaminating organisms is the manure on the teat cups. These systems in which the cleaning solutions move in the same direction as the milk flow (from teat cups to receiving can) will spread those organisms over all the product-contact surfaces of the machine. The reverse-flow system which passes cleaning solutions in the opposite direction, overcomes this problem.

ATTACHMENT OF BACTERIA TO EQUIPMENT SURFACES

An organism may attach to a surface of equipment by one of the three mechanisms below:

1. Attachment directly to the equipment surface;
2. Attachment to soil which is attached to the surface; and
3. Incorporation in a soil mass which,in turn,is attached to a surface.

Examination of the scanning electron micrographs (Figures 2.5-5a, 2.5-5e and 2.5-7c) showed that most bacteria were attached directly to the bare substrate left exposed between adhering soil particles. When the soil level was high, particularly after the milk soiling, the organisms appeared to be embedded with either a thick (Figure 2.5-4b) or a thin covering of soil (Figure 2.5-3b). The difficulty of determining whether the sheetlike substrate under the particulate

soil was truly the surface of the steel slide, or a uniform covering of soil over that surface, makes it difficult to assess the importance of mechanism 2 with regard to the attachment seen here.

Zobell (1943) observed that firm attachment of bacteria to surfaces required an incubation period of 6 - 12h, this being the time required for the organisms to synthesise the extra-cellular polysaccharide by which the cells are attached to the substrate (Marshall et al. 1971b). This requirement for incubation before irreversible attachment took place was shown here by E. aerogenes, the only organism studied which produced extra-cellular polysaccharide. In attachment to both glass in a soiling solution of skim milk (Figure 2.1-7a) and to stainless steel immersed in milk this organism showed an attachment pattern which involved a time interval of 4 - 6h before attachment became apparently irreversible (cf Figures 2.3-6a, 2.3-6c). However, E. coli adhered immediately to the glass surfaces and further incubation did not greatly increase the numbers attached (Figure 2.1-7a). The other tested organisms showed some change with incubation time intermediate between that of E. coli and that of E. aerogenes.

The experiments reported here illustrate the importance of the organism and the surface on the rate of attachment.

1. Figure 2.1-7a shows the variation between the 5 species in the numbers of organisms attaching to glass. The numbers ranged from 160 per slide for S. faecalis to 300,000 per slide for P. aeruginosa after 12h exposure.

   On the surfaces of teeth it was found that some organisms do not adhere directly to surfaces, but attach to other bacteria which are already attached (Gibbons & van Houte 1973; Russell & Coulter

1977). This was not observed in any of the scanning electron micrographs in this study, probably because the numbers of attached organisms were extremely low compared to the numbers present on tooth surfaces.

2. The numbers of bacteria attached to surfaces after soiling was limited in the simulator experiments by the number of "attachment sites" available. This number increased as further soil was deposited on the surface (cf System A.4 with A.1, A.2, A.3 in Figure 2.2-6a). As a result the numbers of bacteria attaching at any soiling (Figure 2.2-6b) were a reflection of the numbers present on the surface before soiling. The number of attachment sites was related to the organism; S. faecalis attached in high numbers to steel, but only in low numbers to a deposited milk soil, whereas E. aerogenes attached in low numbers to steel and high numbers to a deposited milk soil Figures 2.3-6a, 2.3-6c).

The literature review revealed that many other factors also influence attachment.

1. Organisms in the logarithmic phase of growth attach in greater numbers than those in the stationary or death phase (Fletcher 1977).
2. The numbers of organisms which attach increase in proportion to the numbers in suspension (van Houte & Green 1974).
3. The rate of attachment increased with both the temperature and time of exposure to the organisms (Fletcher 1977).
4. The rate of attachment was also affected by the pH and electrolyte concentration of the suspension (Fletcher 1977).
5. In a number of environments an organic layer is the primary

adherent to the surface. On teeth it is salivary glycoproteins (Gibbons & van Houte 1975) and in the marine environment it is comprised primarily of proteins which can either facilitate or impair the attachment of bacteria (Fletcher 1976). Adhesion of bacteria to organic layers may also occur in dairy environments, as Maxcy (1973) found the development of a yellow "milkstone" with P. aeruginosa required a pre-soil of phospholipid.

The attachment of bacteria to surfaces is of great importance ecologically. It permits the bacteria to colonise sites where fluid flow would otherwise remove them (Gibbons & van Houte 1975). Consequently, there is great selection pressure on the organisms for the ability to attach. This ability may be the most important characteristic in determining the ability of an organism to colonize a site. There will be different micro-environments within the milking machine, each with its characteristic pH, redox potential and nutrient supply (Gibbons & van Houte 1975). This was demonstrated by the differences in the microflora developing on rubber and steel surfaces, those present on different individual milking machines in the field experiment and also those obtained with the different cleaning systems (Table 2.6-3).

The attachment of bacteria to surfaces has the additional advantage of locating the bacteria at the site of maximum nutrient concentration which enhances the organisms' chances of survival (Corpe 1974). The behaviour and metabolism of organisms alter when they become attached, which is shown by their reduced multiplication rates, thickened cell walls and large amounts of intracellular polysaccharide (van Houte & Saxon 1971).

DEPLETION OF BACTERIAL NUMBERS BY COMPONENTS OF THE CLEANING SYSTEM

Observed Effects of System Components

An important function of the cleaning system is to minimise the numbers of viable organisms that are removed from the equipment surface into the milk. All methods of reducing those numbers will make a contribution to limiting contamination of the milk. The main methods by which bacteria are controlled are:

1. Physical removal of attached bacteria (detergency);
2. Killing the bacteria (sanitizing); and
3. Inactivation, but not killing the bacteria (bacteriostasis).

The influence of the individual components of the systems on bacteria attached to surfaces has already been discussed in detail in Section 3.1.1. This discussion may be summarised as follows.

Detergents were usually found to be the single most important component of the system, and this contribution could be further increased by using a combined detergent-sanitizer. The performance of the detergent was inversely related to the amount of soil on the substrate, and could be improved if a post-milk (pre-detergent) rinse was used.

The contribution of the iodophor sanitizer to the bacterial control achieved by the whole system ranged from insignificant, to being the most effective single component. The numbers of surface bacteria killed by a sanitizer were influenced by the type and formulation, temperature, concentration and contact-time of the sanitizer. The surface being sanitized and the soil on that surface both influenced the bactericidal control achieved. Of the sanitizers applied for short contact-times the post-wash application resulted in slightly better bactericidal activity than did the pre-milk application.

Periodic application of a sanitizer had a considerable, but transient, influence on the surface flora.

Rinses had little influence on the numbers of surface organisms except when the rinse water was contaminated. However, the post-milk rinse did improve the bacterial control achieved by the following detergent.

Bacterial Injury

It is possible that,in the environments resulting from the application of a cleaning system,many bacteria will be physiologically damaged (by "stressors") but still retain the capability of recovering once conditions again become suitable. They may regain the ability to initiate growth and cell division (Busta 1976). Many factors are capable of imposing such physiological stress during the contamination cycle; osmotic stress by water rinses, thermal stress by hot sanitizing solutions, chemical stress by detergents and sanitizers, and the nutritional stress resulting from being deposited onto clean surfaces. Any of these stressors may injure a bacterium. Such injured bacteria may survive if they subsequently contaminate the milk as they will then be provided with the "unrestrictive" environment which permits recovery and growth.

No attempt was made in these studies to measure the extent of bacterial injury which resulted from the cleaning systems applied. However, the implications of this injury cannot be overlooked as injured bacteria are capable of repair and toxin production (Collins-Thompson et al. 1973). As the inhibiting agents in the selective media normally used for enumeration prevent the growth of injured bacteria, the viable numbers of a particular species or group of

bacteria will be underestimated (Speck 1970). Consequently, the efficiency of any particular cleaning system, or component of a cleaning system, will be over-estimated when measured in terms of the reduction in bacterial count, as will the bacteriological quality of the milk (Speck 1970). The repetitive application of sub-lethal stressors can be an important mechanism for selecting those bacteria which are resistant to that stressor (Busta 1976).

DEVELOPMENT DURING INTERCYCLE PERIOD

After a milking machine has been cleaned there is a rest (intercycle) period of 8-14h before the next milk soiling. This rest period is the only opportunity for bacteria on the equipment surfaces to grow. In all of the simulator experiments performed there was no significant growth during the intercycle period (Figures 2.2-8b, 2.3-9b, 2.3-9d, 2.4-10b and 2.4-10d).

Factors Inhibiting Intercycle Growth

From a bacteriological-control viewpoint it is desirable to prevent growth during this period. For this reason it is necessary to understand which factor or factors in the micro-environment of these bacteria is preventing their growth. There are four main possibilities; nutrition, physical conditions, chemical inhibition and biological interaction.

Unfortunately there has been very little work done on the nutrient properties of milk soil. Barnhart et al. (1970b) and Maxcy (1971) found that a milk film would support growth of many bacteria as long as the water requirements of the organism were satisfied. The organism which grew included streptococci, micrococci, Gram-positive non-sporeforming rods, coliforms, and Gram-negative

non-proteolytic rods. Also, many bacteria are able to survive a considerable period of starvation (Davies 1971). In the early cycles of most cleaning systems used here it was possible that there were insufficient nutrients present for growth but with many systems there was a copious visible soil in later cycles (e.g. Figures 2.2-1, 2.4-1a). Thus, it is likely that there were, at some time, sufficient nutrients present for the growth of at least one of the three dose organisms used (S. faecalis, E. coli and E. aerogenes).

In a number of systems the residues of the final solution applied before the intercycle period remained on the surfaces and increased the proportion of bacteria killed during the intercycle period, compared with the proportion of bacteria killed by those systems in which water was used as the final solution. This occurred in the depletion study (Section 2.2) in which System A.2 (no post-wash rinse) had the alkaline detergent as the final solution (Figure 2.2-8b). In system B.3 (post-wash sanitizer) the final solution of an iodophor had a similar effect (Figures 2.3-9b,-9c). This also occurred in Systems C.3 and C.4 (Figures 2.4-10b,-10d). However, in those systems in which the final solution used was water, this residual effect was eliminated. In such a situation inhibition by detergent and sanitizer chemicals would not appear to be a significant factor in preventing growth during the intercycle period.

The scanning electron micrographs taken at the completion of the intercycle period on stainless steel, rubber, and glass surfaces show that the organisms were very widely distributed. (Figures 2.5-3a, 2.5-4a, 2.5-5a, 2.5-6a, 2.5-7 and 2.5-8). This distribution would have prevented any interaction between organisms.

In the simulator experiments the slides were maintained at 30°C

during the intercycle period. As this is a temperature at which all of the dosing organisms grow well (Table 2.1-3), it is unlikely that temperature was the factor which prevented growth.

The other major physical requirement for growth is sufficient available water. To grow successfully, bacteria require available water levels (Aw) in their media ranging from 0.83 to almost 1.00 (Rose 1976). This is equivalent to relative humidities of from 83% to nearly 100% assuming that the medium is in equilibrium with the atmosphere. The relative humidities applied during the simulator experiments were 80% (depletion study-Section 2.2), 85% (timing of sanitizing study - Section 2.3) and 90% (complementary cleaning experiment - Section 2.4). Thus, it ispossible that the available water in the milk soil was insufficient for growth of the organisms used.

As the deposit on a surface increases in thickness over many cycles the time required for the water in the soil to equilibrate with the atmosphere can increase from a few minutes to many hours (Barnhart et al. 1970b). Thus, as the soil accumulates there will be an increasing period during which the soil contains sufficient water to permit bacterial growth. Initially this period may be less than the duration of the lag phase of growth, but eventually growth will occur (Barnhart et al. 1970a). Protected sites ("harbourages") may be present on equipment surfaces (e.g. corrosion pits, surface cracks in rubber) where there may be a relatively large amount of soil. Such deposits may have only a small proportion of their surface exposed to the atmosphere. Consequently, the rate at which they lose water to the atmosphere will be much smaller than thin films spread on flat surfaces.

The electron micrographs (Figures 2.5-3 to 2.5-8) show that

soils on the surfaces of the slides in the simulator experiments were very thin films, which were probably ineffective at maintaining the available water at the level necessary for the growth of the dose organisms used. However, it can be seen from Figures 2.2-8b and 2.3-9b,-9c that, in many systems, the reduction in numbers during the intercycle period was approaching, or had reached zero. If the same trend in numbers was maintained growth would have occurred had the experimental period been extended.

Microflora of Equipment Surfaces

The proportions of groups of bacteria within the microflora on surfaces were determined in two experiments. These were reported on for four systems evaluated by the simulator (Table 2.5-3) and the same four plus an extra system evaluated by field experiment (Table 2.6-3). The data for streptococci and coliforms for the simulator experiment must be examined carefully as these were the organisms used to dose the milk.

Both tables show the influence of the surface upon the microflora. With System D.1 (ABW) in the simulator the steel surfaces were dominated by micrococci, while the rubber surfaces were dominated by flavobacteria and pseudomonads. Similarly, in the milking machines cleaned by the warm-iodophor system the microflora of the steel surfaces was dominated by yeasts and coliforms, while the rubber surfaces were dominated by bacilli and streptococci, as well as coliforms.

In the field the situation is more complex. The microflora is known to vary considerably from site to site within milking machines. This is a result of the different surface materials

and uneven distribution of soil, temperature and available water throughout the machine (Twomey 1975).

Druce and Thomas (1972) observed that in 86% of their samples of the microflora of milking machine surfaces one organism constituted more than half of the population. This observation was confirmed in the experiments reported here; all of the isolates in the simulator experiment had a single dominant organism (Table 2.5-3), as did 12 of the 16 isolates in the field experiment also (Table 2.6-3). However, the organisms which were dominant depended on the farm investigated and the cleaning system used.

The variations imposed by the cleaning systems on the environment of the bacteria made the system the most important factor influencing the proportions of organisms in the microflora (Table 2.6-3).

1. The microflora associated with the ABW system consisted primarily of Bacillus spp., with some micrococci, Lactobacillus spp. and coliforms. Of these both the Bacillus spp. and coliforms may cause problems regarding milk quality (Thomas et al. 1963).
2. The microflora associated with the cold iodophor system was dominated by coliforms and Streptococcus spp., with a few Bacillus spp., micrococci and Lactobacillus spp.. In the practical situation, a microflora of this composition may give significant problems in terms of milk quality as the coliforms and Streptococcus spp. produce acid and are proteolytic (Thomas et al. 1963).
3. The microflora associated with the warm iodophor system was dominated by Bacillus spp., Streptococcus spp. and yeasts, some micrococci and Lactobacillus spp.. In the practical situation yeasts, Bacillus spp. and Streptococcus spp. can give milk quality problems as they are acid-producers (Thomas et al. 1963).

4. The microflora associated with the cold alkali system was dominated by micrococci and Bacillus spp., with a few Streptococcus spp., Lactobacillus spp., flavobacteria and other Gram negative rods. Such a microflora is not generally associated with problems regarding milk quality in the dairy industry (Thomas et al. 1963).

CONTAMINATION OF MILK FROM THE EQUIPMENT SURFACE

Some of the viable organisms present on the surfaces of a milking machine immediately before milking may be removed into the milk, effecting contamination. This is a little-studied component of the contamination cycle. It is not a simple process of deposition, growth and recovery: Maxcy (1969) reported that "harbourages" greatly influenced the result. Twomey and Crawley (1969) confirmed this when they observed that the release of thermoduric bacteria from milking machines was discontinuous, the daily variation being ten-fold.

The number of organisms effecting contamination would depend upon the strength of attachment of the organism to soil or substrate, and also upon the physico-chemical nature of the recovery solution (milk) and the physical conditions (turbulence etc.) under which it was applied. Little information has been obtained on this process in this work, but the data in Table 2.5-2 suggest that the pre-milk count must exceed $\log_{10}$ 1.5 per slide before contamination occurs, indicating that only a very small proportion of the surface organisms ($\sim$1%) became contaminants.

3.2.2 IMPLICATIONS OF THE DATA

This study of the cycle of bacterial contamination on milking machine surfaces has a number of implications:

1. Under the experimental conditions in the simulator the milk-soiling

was the only component which increased the bacterial numbers. Thus, the contamination cycle was a straightforward deposition/depletion/contamination process. However, in the practical situation, this would be complicated where there are other sources of contamination (e.g. cleaning solutions, atmosphere), or where the intercycle conditions are suitable for bacterial growth. This latter condition will arise if there are protected sites, (harbourages) where soil can accumulate, and the rate of water loss from the soil to the atmosphere is limited.

2. The nature of the environment during the intercycle period greatly influenced the total bacterial control achieved. Thus, in the practical situation, it is absolutely essential that the surfaces be in an environment which limits, and preferably eliminates, bacterial growth. This can be achieved by a number of techniques;
   (a) Desiccation - by aeration, or immersion in a non-aqueous solution (e.g. ethanol),
   (b) Temperature (>80 or $<0^{\circ}C$),
   (c) Chemically (sanitizer in contact for whole period), or
   (d) Physically (ultrasonic, electrical or similar treatment).

   Thus, the treatment during the intercycle period must be considered to be an integral component of the cleaning process. If the cleaning solutions can be applied to the equipment surfaces for extended periods, the "soak" application of a detergent-sanitizer provides a long contact-time for the sanitizer to act, and also solves the problem of how to prevent growth in the intercycle period.

3.2.3 FURTHER WORK REQUIRED

The experiments performed have demonstrated the existence of a

contamination cycle for bacteria on the surfaces of food equipment. The results indicate our ignorance on this important topic. Some of the areas requiring further investigation are:

1. Each step of the contamination cycle should be studied in detail in the laboratory as units and also as phases of the complete cycle; deposition, attachment, depletion, development and contamination. This work should be performed on the surfaces used in the food industry, soiled with appropriate food residues.
2. The operation of the contamination cycle should be examined under field conditions.
3. Bacteria do grow on food equipment in protected sites (harbourages) under specific conditions. The ecosystem of these harbourages requires study including such aspects as; the effect of the composition and depth of soil on the bacteria (TEM and ZEMs), the effect of the bacteria on soil composition and the succession of organisms within the bacterial community.
4. The most important phase in the contamination cycle is attachment. If the primary colonizing organism can be prevented from attaching and metabolizing on the surface, the succession of organisms which follow may be unable to colonize the surface. In a marine environment a non-lethal bacterial repellent was capable of greatly reducing the attachment of a mobile pseudomonad, which was the primary colonizer. This interfered with the succession of organisms and consequently the total mass of bacterial film attached to the surface was greatly reduced (Chet et al. 1975). This type of attachment control may be achieved in the dairy situation by careful selection of the material and finish of equipment surfaces, and application of either repellent (for motile bacteria), or inhibitor

(for non-motile bacteria).

5. The factors affecting the need for, and methods of, periodic cleaning require definition.
6. The relative importance of killing and removing bacteria as control methods by each system component requires examination.

3.3 EVALUATING THE PERFORMANCE OF CLEANING SYSTEMS

The literature review on assessing the performance of cleaning systems (Section 1.7) showed that there are three types of performance parameters:

1. Economic performance of the system. The review showed that this was the ultimate measure of system performance, but that it was rarely used in research. Although the experiments reported here aimed at reducing the costs of cleaning through a lower requirement for thermal and kinetic energy, the overall economics of the systems were not calculated.
2. Assessing the quality of the milk produced. To obtain maximum economic return for the milk produced it must meet certain physical, chemical and biological quality criteria. These were assessed in the field trial (Section 2.6), where the milk was routinely subjected to: sediment test, freezing point test for added water, methylene blue and nitrate reductase keeping quality tests, and counts for thermoduric and coliform bacteria. The information on the significance of means presented in Table 2.6-1 shows that, parameters assessed on the milk (coliform count and thermoduric count for the whole machine) were much less sensitive to differences in cleaning system performance than the parameters assessed on the machine surfaces (deposit, surface TYGA and VRBA swab grades).

   In the simulator experiments the deliberate dosing of the milk with marker organisms precluded the use of milk quality changes as parameters of system performance. An indication of the biological quality was obtained in the simulator experiment described in

Section 2.5, where the numbers of organisms contaminating the milk from the equipment surfaces were determined.

3. Assessing the condition of the surfaces of the equipment. Both the physical (soil) and biological condition (microflora) of the equipment surfaces were determined in both simulator and field experiments. As shown above, these were the most sensitive indices of system performance.

3.3.1: SIMULATOR ASSESSMENT

In determining the experimental program a choice had to be made between replicating treatments between simulator experiments to assess reproducibility, or using different treatments in each experiment to obtain the maximum amount of new information. The massive amount of time and effort required to complete a simulator experiment limited the number of simulator experiments which could be conducted to four. Because of this small number of experiments, different treatments were conducted in each simulator run to obtain the maximum possible amount of new information.

The simulator technique has the advantage of accurate control over the physical, chemical and biological conditions of the experiment. In this work the chemical composition, temperature and bacterial content of all of the soiling and cleaning solutions were controlled and monitored. Although not demonstrated statistically in these experiments, this control over conditions,and the fact that the four test cells were soiled with a common solution, had the same air supply and temperature conditions and were running simultaneously, must all have improved the reproducibility of the technique. Also, the mechanical control of the simulator operation reduced the operator variability

in the evaluation of the performance of the system.

The levels at which some factors were applied were selected to place the greatest possible load upon the cleaning system; the numbers of bacteria in the milk were fairly high (10^6/ml) to accelerate soiling; the hardness of the water was also fairly high for NZ conditions (200 mg/l total hardness as $CaCO_3$) in order to place a greater cleaning burden on the detergents and sanitizers. This accelerated the rate of soil and bacterial development on the test pieces and consequently increased the rate of experimentation (cf simulator 35 days : field trial 70 days). In many simulators, where the amount of deposit is the only cleaning parameter assessed this rate can be further increased by reducing the intercycle time (e.g. Whittlestone et al. 1963; Dunsmore et al. 1977a). As the intercycle time is the main period for bacterial growth this technique cannot be applied where bacterial development is being assessed.

Only one simulator study (Twomey, 1975) has previously examined any bacteriological effects of cleaning. That study examined the ecology of coryneform organisms. Thus, the work reported in this thesis is the first in which the bacteriological performance of cleaning systems has been thoroughly examined. This was facilitated by the test cell design, in which the lack of solution turbulence permitted large numbers of test pieces (130) to be treated equally. The features which were incorporated into the simulator design to permit bacteriological evaluation were:

1. Filtration of water supply;
2. Filtration of all air inlets to machine, and also of air circulating through the test cells;
3. Control of humidity and temperature of air circulated through test

cells;

4. Routine washing and steaming of milk dispenser system and solution inlet manifold;
5. Elimination of pumps by gravity feeding all solutions; and
6. Single use of all cleaning solutions.

The simulator technique also has a number of disadvantages. Simulators are inherently complex pieces of apparatus, and the machine used in this work was more complex than any previously used. As a result, the programming and control systems were highly sophisticated, which made them expensive and sometimes unreliable.

Although simulators permit the evaluation of the equipment-surface parameters of cleaning, they are unsuitable for assessing milk quality, or the overall economics of cleaning. Also, the fact that the experimental situation is perforce confined to a few substrates, a specific water quality, a few organisms, and also that only a few parameters are assessed,mean that the results can be somewhat limited in their application.

The final disadvantage with the simulator technique is that relationship between the results obtained by both simulator and field assessment must be established. In the experiments reported in this thesis four cleaning systems were evaluated by both field and simulator experiments. The ABW, triple (T), cold-iodophor (CI) and cold-alkali (CI) systems of the field trial (Section 2.6) were also examined in the simulator experiment in Section 2.5. The results of assessment of related parameters by both techniques are presented in Table 3.1-1. The means and the significant differences for the field trial were obtained from Table 2.6.1. The means and significant differences for the simulator experiment were obtained from Table

2.5-2. The performance of each system is ranked from high value to low (a - d) and statistical differences ($p < 0.05$) are denoted by dissimilar ranking letters. As there were no suitable intercycle counts on steel surfaces from the simulator, the post-milk soiling counts have been used as measures of the bacteriological performance of the systems.

The relationships between the simulator and field results for deposit and bacterial counts were very poor. This was probably caused by the different application conditions of the cleaning solutions; in the milking machine a sufficient flow rate was maintained to achieve turbulence and fresh solution was passed over the surface continually, while in the simulator the test pieces were soaked without any turbulence and the same solution was in continuous contact with the surfaces. This result indicated that there was a very significant interaction between the physical and chemical factors affecting the performance of cleaning solutions. Consequently, in order to be directly comparable with field conditions, it is most important that the simulator duplicate the physical manner by which the solutions are applied.

3.3.2: FIELD ASSESSMENT

The field trial has the advantage of being the "ultimate test" of a cleaning system (Davis 1956a) because it is conducted under practical conditions. These conditions include the physical application techniques, the natural soiling conditions, the water quality, the different substrates, the natural micro-organisms and the people applying the system.

It also has the advantage of permitting evaluation by all three

Table 3.3-1: The performance of 4 cleaning systems examined by field trial and simulator techniques and assessed by 4 parameters

Data	Cleaning system	Method of evaluation			
		Field		Simulator	
		Amount	Ranking	Amount	Ranking
Soil on steel ($\mu g/cm^2$)	ABW	3.2	c	464.5	a
	T	8.3	b	58.8	b
	CI	6.8	bc	51.8	b
	CA	13.7	a	26.0	c
Bacteria - surface total count	ABW	1.37 grade	b	log 3.8	c
	T	1.61 grade	b	log 6.1	a
	CI	1.44 grade	b	log 6.6	a
	CA	2.08 grade	a	log 5.7	b
Bacteria - surface coliform count	ABW	0.39 grade	b	log 3.0	c
	T	0.50 grade	b	log 2.2	d
	CI	0.53 grade	b	log 5.0	a
	CA	1.05 grade	a	log 4.0	b
Bacteria - milk coliform count	ABW	log 2.01	ab		
	T	log 1.74	b		
	CI	log 2.24	a		
	CA	log 2.38	a		

types of assessment; economic, product quality and condition of the equipment surface. Of these, only the last two were obtained in the field trial reported in Section 2.6, but information for an economic analysis could have been easily obtained. The correlations between the various parameters of the performance of milking machine cleaning systems are presented in Table 3.3-2. The amount of deposit appeared to influence the "coliform" grade of the machine surface and also the coliform count of the milk, but did not affect any other parameter. The high correlation between the VRBA grade of the machine surface and coliform count of the milk indicated that the machine surfaces were a significant contributor of coliform organisms to the milk. The presence of these coliforms was also detected by the nitrate reductase test ($r = 0.519$). The thermoduric count of the milk was not significantly correlated to any other parameter. There was a high correlation ($r = 0.802$) between the VRBA and TYGA grades of machine surfaces. The bacterial counts of the milk and nitrate reductase test were performed because they are mandatory or advisory measures of milk quality. The deposit data was obtained to determine the detergency of the system, and the machine surface grades were used as a measure of the sanitizing performance of the system. Examination of Table 3.3.2 shows that the nitrate reductase test was highly correlated to surface bacterial grades and milk coliform count. This test could be omitted, but all of the remaining parameters were measuring something different about the cleaning system performance. These other five parameters are all of practical value and should be used in any further field experiments.

A further advantage of the field trial is that it permits the examination of the interaction between the equipment design and the

Table 3.3-2: Correlations between parameters of cleaning performance obtained in the field trial

Parameters	Data	Parameters				
		Deposit	Milk coliform count	Milk thermoduric count	Milk nitrate reductase test	Surface TYGA grade
Milk coliform count	r*	0.244				
	df**	21				
	r0.05***	0.423				
Milk thermoduric count	r	0.005	0.033			
	df	21	57			
	r0.05	0.423	0.250			
Milk-nitrate reductase test	r	0.251	0.519	0.036		
	df	21	57	70		
	r0.05	0.423	0.250	0.232		
Surface TYGA grade	r	0.105	0.259	0.075	0.501	
	df	21	57	70	73	
	r0.05	0.423	0.250	0.232	0.230	
Surface VRBA grade	r	0.455	0.378	0.129	0.588	0.802
	df	21	57	70	73	73
	r0.05	0.423	0.250	0.232	0.230	0.230

* r = correlation coefficient

** df = degrees of freedom

*** r0.05 = correlation coefficient required for significance ($p < 0.05$)

cleaning system. With milking machines this is expressed in the variation in cleaning performance at different sites within the machine. In the field experiment all machines were swabbed at ten sites and the swabs plated onto two media; TYGA and VRBA. The mean swab grades for the various sites and whole machine for the five systems are presented in Table 2.5-2. The correlation coefficients between individual sites and the whole machine for each medium, and the significance of any differences between systems, are presented in Table 3.3-3. The TYGA and VRBA grades all had significant correlation coefficients, ranging from 0.478 to 0.816.

If the number of sites sampled during the field trials with milking machines were to be reduced, the selection should include those sites which correlate well with the whole machine and permit significant differences to be obtained. The sites which satisfy these criteria are the teat cup liners, end of milk line and milk cooler. The selection should also include swabs of at least one of each different type of material; in this case rubber (liners), stainless steel (end of milkline and cooler) and plastics (sight glasses).

3.3.3: IMPLICATIONS OF THE DATA

The information presented has a number of implications:

1. There is a very significant interaction between the chemical and physical factors in the cleaning situation. All simulator trials must be performed under the appropriate physical conditions.
2. This work has required the development of a simulator design and associated techniques to permit the bacteriological assessment of cleaning systems. These techniques create the opportunity for the routine evaluation of systems and system components by bacteriol-

Table 3.3-3: Correlations for bacterial swab grades on two media between individual sites of milking machines and the whole machine

Machine site	MEDIA			
	TYGA		VRBA	
	r*	Sign.**	r	Sign.
1 & 2 - teat cup liners	0.750	***	0.777	*
3 & 4 - dropper tubes	0.785	NS	0.793	*
5 & 6 - sight glasses	0.478	NS	0.619	NS
7 - end milkline	0.619	**	0.598	***
8 - receiving can	0.633	NS	0.614	*
9 - milk cooler	0.816	***	0.782	***
10 - delivery tube	0.614	NS	0.689	NS
Whole machine	-	***	-	**
r. required for significance ($p < 0.05$)	0.230	-	0.230	-

*r = correlation coefficient between machine site and whole machine.

**Sign. = Significance of differences between cleaning systems at each site, obtained by analysis of variance.

ogical as well as the physical and chemical methods used previously.

3. Despite their advantages and disadvantages, both the simulator and the field evaluation techniques have their place in hygiene research and development. Any radical new idea (e.g. cold cleaning) should be evaluated by field trial and, if found satisfactory, further development work can be quickly performed by simulator trial as a prelude to final proving trials in the field (presuming a good correlation between field and simulator). Minor modifications to an already established system may be assessed by either simulator or field trial.

3.3.4: FURTHER WORK REQUIRED

The work described has demonstrated a need for further study of simulator assessment. The test pieces traditionally used in simulator evaluation are planar. In these experiments these surfaces have not been able to support bacterial growth, despite an abundance of nutrients, appropriate temperature for growth and high relative humidity (see Section 3.2). In the milking machine, growth occurs not on the planar surfaces, but in cracks and crevices (harbourages). The possibility of using surfaces with standardised "harbourages" in simulator experimentation should be examined.

3.4 THE DESIGN OF CLEANING SYSTEMS

In the literature review (Section 1) the cleaning of food equipment was shown to be a complex interaction of many physical, chemical and biological factors. It also showed that, while there has been some empirical progress in the performance of cleaning systems, there has been little study of system design. Consequently, no 'perfect' cleaning system has been introduced. This section will discuss many of the factors which influence the design of cleaning systems for all food equipment, but will use milking machines as examples.

To be successful a cleaning system must meet certain performance criteria. For most products these criteria are stated in terms of the physical, chemical and microbiological quality of the product. Davis (1956) defined the "minimum efficiency level" as that level of operation which could always be relied upon to meet those quality criteria by a safe margin. From a practical viewpoint the best cleaning system is that which reaches the "minimum efficiency level" for the minimum cost and minimum labour requirement.

3.4.1 FACTORS INFLUENCING THE DESIGN OF CLEANING SYSTEMS

The characteristics of each cleaning situation will largely determine the design of the cleaning system which will give the best results. The characteristics which are important include nature of the surface, the geometry of the equipment to be cleaned, the soil, detergent and sanitizer compositions, intercycle conditions, product-criteria, economics and resources. The implications of each of these characteristics in terms of the physical, chemical and biological aspects of the system and also system design are presented in Tables

3.4-1 and 3.4-2.

The interactions between the factors are extremely complex, and will vary with each combination of equipment and soil. An understanding of the way in which the factors interact can be obtained by examining the milking machine as an example. Most of the information has been presented previously and will be documented solely by referring to the appropriate section of the literature review or general discussion. The information is discussed in sections under headings taken from the tables.

SUBSTRATE

The substrates (Table 3.4-1) on a milking machine are the product (milk) contact surfaces which include stainless steel, glass, rubbers and plastics (refer section 1.2). All of these can be sanitized by heat, but stainless steel and rubber should not be in contact with oxidising acids (except nitric acid) or chlorine solutions for long periods. Different detergent formulations are required for plastics material than are conventionally used for other materials. As soon as items made of these materials show signs of surface deterioration, the components should be replaced.

EQUIPMENT TO BE CLEANED

The pipeline milking machine has a high surface/volume ratio (Table 3.4-1) which permits solutions to flow turbulently through the equipment (refer Section 1.4). This high ratio also indicates that the milking machine may contribute significant numbers of bacteria to the milk as it passes through. The pipeline system lends itself to the mechanical application of cleaning solutions and this may be mechanised. The intricate shapes create sites protected from the clean-

Table 3.4-1: Implications of the substrate, equipment to be cleaned and soil, on the characteristics of a cleaning system

Cleaning situation		Implications of cleaning situation			
Component	Characteristics	Physical	Chemical	Biological	Nature of cleaning system
Substrate	Product-contact surfaces; composition and finish.	Limitations on use of heat or other energy sources.	Limitations on corroding or degrading chemicals, limitation of contact-time.	Affect microbial attachment; absorption of micro-organisms (MO's) and nutrients by porous surfaces	-
	Non-product-contact surfaces touched by system components	Limitations on use of heat or other energy sources.	Limitations on corroding or degrading chemicals	-	-
Equipment to be cleaned	Surface/volume ratio	- Affects solution application method; low ratio- solution flow, high ratio- spray/foam methods.	- Affects foam characteristics of solutions.	Affects significance of surface MO contamination in products	
	Design of equipment:				
	pipeline,	- Use turbulent solutions.	- Low foaming.	- Not exposed to environmental contamination. Product flow effectively incorporates MO contaminants.	- Mechanised cleaning systems.
	open vessel,	- Use sprays/falling film or foam.	- Low or high foaming.	- Exposed to environmental contamination.	- May require pre-product sanitizing.
	complex.	- Use sprays or manual.	- pH limitation on manual cleaning solns.	- Personnel contamination from manual handling.	- Manual system.
	Volume of cleaning solutions	- Dilution of product by water from rinse residues.	- Contamination of product by sanitizer residue if non-rinse. - Contamination of cleaning soln. by residues of previous soln can cause inactivation.	- Sanitizer residues may affect consumer health, manufacture of fermented products. - Inhibition of sanitizer performance.	- If necessary rinse out sanitizer or detergent-sanitizer. - If necessary put rinses between detergents and sanitizer.
Soil	Frequency of soiling	-	-	Affects growth period for MO's.	Pre-product sanitizing not required at frequency > 1/24h, simple routine system with complex periodic system with frequency < 1/24h.
	Adherence of soil:	Energy requirement for cleaning;	Chemical energy required for cleaning;	Establishment of surface colonies;	System complexity alters;
	- low,	- low-soak in manual cleaning,	- low - simple solutions only,	- difficult,	- single detergent and infrequent periodic clean may suffice,
	- medium,	- moderate-turbulence and heat,	- moderate,	- moderate,	- single detergent and frequent periodic clean may suffice,
	- high.	- high - impingement/turbulence and heat.	- high - strong acids/alkalis and solvents.	- easy	- double detergent system with periodic clean may be required.

ing solutions where soil may accumulate and support bacteria growth. The closed nature of the apparatus limits the opportunity for aerial contamination of product-contact surfaces. Poor drainage of cleaning solutions can raise the humidity to levels where bacteria can grow. If the undrained solution is a sanitizer there may be potential problems with human health (Dunsmore et al. 1977a) or cheese manufacture (Dunsmore, et al. 1978). An undrained solution may also inhibit the activity of other cleaning solutions if inadequate volumes are applied, or the solutions are recirculated (Dunsmore et al. 1977b). The necessity to remove a sanitizer or detergent-sanitizer is dependent upon the time within the cycle at which it is applied (post-product wash or pre-product rinse) and the chemical nature of the solution used.

SOIL

The 12 hourly soiling frequency (Table 3.4-1) limited the growth period and prevented any significant bacterial growth during the intercycle period (Section 3.2-1). Therefore, if the post-washing treatments are satisfactory, no pre-milk sanitizing should be required. The soil adherence is relatively low, making bacterial colonisation of the surface difficult and the use of low energy cleaning systems a possibility.

CHEMICALS

The soil (Table 3.4-2) may be amorphous or crystalline and is largely a mixture of fat, protein, calcium phosphate and other minor organic components (Section 1.3). It can protect the bacteria from the cleaning system and provide satisfactory nutrients for bacterial growth. This type of soil can be cleaned by very acidic detergents

Table 3.4-2: Implication of chemicals, intercycle conditions, product requirements, economics and resources on the characteristics of the cleaning system

Cleaning situation		Implications of cleaning situation			
Component	Characteristics	Physical	Chemical	Biological	Nature of the cleaning system
Chemicals	Chemical nature of soil	-Affects thermal conductivity, Affects structure.	-Defines nature of detergents used. Affects adherence of soil.	-Determines suitability as nutrient for micro-organisms (MO's). Structure affects protection of MO's from environment, Determines MO attachment, Determines efficiency of MO recovery by food.	-Determines the sequence and frequency of acidic/alkali detergents.
	Treatment between soiling and washing	-Affects soil adherence (see Table 3.4-1).	-Affects soil adherence (see Table 3.4-1).	-Affects soil adherence and MO attachment (see Table 3.4-1).	-Affects soil adherence (see Table 3.4-1).
Intercycle conditions	- Temperature, - Time, - Humidity, - Aeration:	-Affect soil adherence (see Table 3.4-1).	-Affect soil adherence (see Table 3.4-1).	-Affect growth of MO in intercycle period and ultimate level of contamination.	- Affects need for pre-product sanitisers. May apply intercycle soln for bactericstatic or bacteriocidal action.
Product requirements		-No physical contamination.	-Within specified composition. No chemical contaminants.	-No pathogenic MO, Limited no. of specified types and total MO's.	-Sufficient efficiency to meet product criteria.
Economics	Cleaning equipment available	-Affects application method.	-Application method affects solns used.	-	-As simple as possible minimise concentrations minimise thermal energy.
	Single use/recirculation or re-use of solutions:	-Affects application method.	-Affects chemicals selection.	-MO's may contaminate re-used solutions.	- Water rinses sometimes re-used. Cleaning solutions often re-used.
Resources	Water		-Water chemical quality affects detergent and rinse performance.	-Contaminated rinse water may contaminate surfaces.	- Post product rinse gives efficient soil removal, Contaminated rinse water precludes pre-product water rinse.
	Chemicals		Limitations on formulation may affect performance.		
	Labour	- Affects complexity of cleaning control equipment.	-Dangerous chemicals should not be used.	-Contamination of product dependent on labour performance.	-Affects complexity of system - simplicity assists reliable performance.

or those ranging from pH 7 to 13. It should be washed off immediately to prevent any ageing, which would greatly increase adherence to the substrate.

INTERCYCLE CONDITIONS

The temperature, humidity and aeration of the 12 hour intercycle period (Table 3.4-2) affect the adherence of the soil in a minor way, but have a major affect on the growth of organisms on the equipment surfaces prior to the next milking (Section 3.2-1). Thus, the intercycle conditions should be controlled to create a microenvironment unsuitable for bacterial growth. This can be achieved by desiccation, temperature or sanitizing. The intercycle period is an integral part of the whole cleaning system.

PRODUCT REQUIREMENTS

Milk quality (Table 3.4-2) is assessed in terms of chemical composition, bacteriological composition and cell count (refer Section 1.7). The cleaning system can affect the chemical composition by adding water or sanitizer residues to the milk. The most sensitive measure of cleaning system performance is the bacteriological quality of the milk. The particular factors in milk quality assessment vary from country to country, or even between dairy factories. Common assessments include numbers of total, thermoduric, psychrophilic, and coliform organisms. Often keeping quality tests such as the methylene blue reductase test replace bacterial counting methods.

ECONOMICS

Economics (Table 3.1-2) provide a strong incentive to simplify milking machine cleaning systems. A two-step hot system such as

acidified-boiling-water (ABW) is a considerable improvement over a four-step hot program such as the Triple system (refer Section 3.1). Similarly, the cold-iodophor system is an improvement over the hot systems, in terms of the cost of installing and operating water heaters, provided that the cleaning performance is satisfactory. Detergents and sanitizers are not currently re-used in milking machine cleaning, but this may occur in the future. Solutions are applied by a wide variety of techniques, including reverse-flow, recirculation and flush methods.

RESOURCES

On the dairy farm the fact that the available water resources (Table 3.4-2) are extremely variable in both chemical and bacteriological quality can be a significant problem. The major chemical problems are acidity, alkalinity, hardness and excessive iron. Some farms have installed water-treatment plants to correct these chemical and bacterial problems but, if not, it is desirable to include a sanitizer in the final solution before milking to prevent contamination occurring (Section 3.2-1). However, this can create some further problems in terms of sanitizer residues in the milk.

The staff involved in milking machine cleaning are usually not trained in the principles of hygiene, and are often poorly instructed in their task. This has led to the recommended cleaning system being arbitrarily altered in many situations, which often causes a reduction in milk quality. Some automated systems have been developed to overcome this problem, but these complex mechanisms can introduce further problems of their own.

3.4.2 DESIGNING A CLEANING SYSTEM

The data obtained in the experiments reported in this thesis, along with previously published information, can be used to design a cleaning system for any situation in the food industry. The information presented in the previous section (3.4.1) described the cleaning situation and drew some conclusions on the possibilities for, or limitations on, system design. This type of information is required for a cleaning system to be designed for any particular situation.

When designing a cleaning system a number of questions present themselves. The most fundamental of these are examined below.

THE CLEANING SITUATION

To design a cleaning system the characteristics of the cleaning situation must be defined,with any particular requirements specified. The example taken here is that of a pipeline milking machine required to be cleaned by cold solutions. The surfaces of this hypothetical machine are taken to be mainly stainless steel, with some rubber and plastics.

HOW MANY COMPONENTS ARE NECESSARY IN THE CLEANING SYSTEM?

The components of a theoretical cleaning system for any piece of equipment are presented in Table 3.4-3. This gives all of the possible steps for a system applied routinely, at every use of the equipment, and an alternative system (Periodic) when a resistant soil accumulates over time. The number of components will be dependent upon the chemistry and adherence of the soil, the frequency of soiling and the method of solution application.

In the case of milking machines the soil is not very adherent if

Table 3.4-3: Possible components of routine and periodic systems for all food equipment.

Frequency	Timing of action	Action
Every soiling	1.1 Post-product	1.1-1 Physical removal of gross soil
		1.1-2 Post-product rinse
		1.1-3 Detergent A (or detgt-san. A)
		1.1-4 Post-detergent A rinse
		1.1-5 Detergent B (or detgt-san. B)
		1.1-6 Post-detergent B rinse
		1.1-7 Sanitizer C
	1.2 Intercycle	1.2 Sanitizer D (or detgt-san D)
	1.3 Pre-product	1.3-1 Pre-product sanitizer E (or detergent-sanitizer E)
		1.3-2 Pre-product rinse
Accumulated soil (periodic)	2.1 Post-product	2.1-1 Physical removal of gross soil
		2.1-2 Post-product rinse
		2.1-3 Detergent PA (or detgt-san. PA)
		2.1-4 Post-detergent PA rinse
		2.1-5 Detergent PB (or detgt-san. PB)
		2.1-6 Post-detergent PB rinse
		2.1-7 Sanitizer PC
	2.2 Intercycle	2.2 Sanitizer PD or detgt-san. PD)
	2.3 Pre-product	2.31 Pre-product sanitizer PE (or detergent-sanitizer PE)
		2.33 Pre-product rinse

washed as soon as milking is completed. Consequently, only one detergent or detergent-sanitizer will be required routinely. The 12-hourly soiling frequency means that there will not be significant growth in the intercycle period. Consequently, the pre-product sanitizer (E) can be eliminated. The post-wash sanitizer may be applied as a short application (C) or for the intercycle period (D). This leaves as possible components 1.1-1, 1.1-2, 1.1-3 or 1.2, 1.1-4, 1.1-7, 1.3-1 and 1.3-2 in Table 3.4-3.

WHAT SORT OF INTERCYCLE TREATMENT CAN BE USED?

The length of the intercycle period, the geometry of the equipment and the environment during the intercycle period all influence the treatment (if any) applied during the intercycle period.

In the milking machine, the equipment can be arranged to hold solutions throughout the intercycle period. The environment within the milking machine is such that growth may occur if there is sufficient residual soil. Thus, control of the atmosphere during the intercycle period is desirable, and this is most conveniently done by filling the machine with an antibacterial solution, rather than by controlling the humidity or temperature within the machine.

If a sanitizing solution is to be placed in the machine for the control of bacteria, the application of a detergent-sanitizer also gives the detergent a long time in contact with the soil which improves its efficiency. This means that (in Table 3.4-3) use of a detergent-sanitizer D eliminates detergents A and B, and that the post-detergent rinses A and B will also not be required. However, the pre-product rinse (1.3-2) will still be necessary in order to remove the residues of the intercycle detergent-sanitizer from the

machine. This reduces the number of components to 1.1-1, 1.1-2, 1.2 and 1.3-2 in Table 3.4-3.

WHAT TYPE OF FORMULATION WILL THE MAIN DETERGENT HAVE?

The formulation of the main detergent will be influenced by the chemical composition and treatment of the soil, the method of, and the temperature at which the detergent is to be applied.

The experimental work described here has shown that in the milking machine the milk can be cleaned by turbulent acidic or alkaline solutions, but only by alkaline solutions in soak situations. As the main detergent is also to be used as a soaking solution during the intercycle treatment it must be an alkaline detergent-sanitizer.

WHAT TYPE OF FINAL RINSE TREATMENT WILL BE REQUIRED?

The final rinse treatment must be a sterile solution - either uncontaminated water, or a sanitizer. If a sanitizer is used it must not leave residues which may be a health or technological problem. Also, the final rinse may be used to complement the detergency of the main detergent.

In the milking machine example discussed so far I have suggested that an alkaline solution be used as the major detergent. An acidic final rinse would therefore complement the cleaning action of the main detergent.

Some sanitizers used as pre-product rinses in milking machines have caused problems with residues in the milk (e.g. iodophors and QAC's). Therefore, a sanitizer must be selected which does not cause these problems.

WHAT SORT OF PERIODIC CLEANING WOULD BE REQUIRED?

The frequency and nature of periodic cleaning of food equipment is dependent upon the nature, rate of deposition and potential as a bacterial medium of the soil which remains following application of the routine system for some time.

In the milking machine to which the above system has been applied, an adherent residual soil consisting primarily of minerals and protein will accumulate. This will be most efficiently removed by a high concentration of acidic detergent at high temperature. The elevated temperatures will also be bactericidal to any organisms within the soil. This main detergent may be followed (after rinsing) by a high concentration of alkaline detergent at high temperature. This should be followed by a hot water rinse in order to remove the detergent-soil residues without recontamination of the machine.

DETAILS OF THE PROPOSED CLEANING SYSTEM

The final procedure is a combination of routine and periodic systems:

Every Milking:

1. Remove gross soil from teatcups with brush in rinse-water (containing non-ionic rinse additive).
2. Flush this cold, non-ionic rinse through the milking machine to waste.
3. Fill the machine with cold, alkaline detergent-sanitizer from a reservoir and leave for complete intercycle period.
4. Pump the detergent-sanitizer back to the reservoir before the next milking;
5. Flush a cold solution of sulphamic acid through the machine to

waste.

Periodically (when the soil accumulation is excessive):

1. Remove gross soil from teatcups with brush and cold rinse-water (containing non-ionic rinse additive).
2. Flush this cold non-ionic rinse through the machine to waste.
3. Recirculate a high concentration of acidic detergent through the machine for 20 min at 80°C.
4. Flush cold tap water through the machine to waste.
5. Recirculate a high concentration of alkaline detergent through the machine for 20 min. at 80°C.
6. Rinse with water at 80°C.
7. Drain and air dry.

The advantages of this system are; no heat required for routine cleaning; no special cleaning apparatus required; re-use of main detergent-sanitizer; efficient deposit control through long contact-time of detergent and complementary detergency; efficient control of bacteria through long contact-time of sanitizer and control of intercycle growth. However, it must be acknowledged that the design of a cleaning system can never be solely a theoretical exercise. Many factors affecting its performance will be unknown and developmental experiments will be required before the system can be adopted in the industry.

4

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