

Bacteriological Control of Food Equipment Surfaces by Cleaning Systems. I. Detergent Effects

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ABSTRACT

A cleaning simulator was used to determine the changes in soil and bacterial numbers on stainless steel surfaces over 36 soiling and washing cycles (each of 12 h) with four cleaning systems. The soiling milk contained 10^6 *Streptococcus faecalis* colony forming units/ml. One system included a pre-milk iodophor rinse (20 C), milk soil (30 C), post-milk water rinse (20 C), alkaline detergent (50 C) and a final water rinse (20 C). The second system was similar, but without the final rinse. In the third system, the post-milk rinse was omitted. The fourth system was similar to the second, except that water at 20 C was substituted for the detergent. The simulator technique proved effective for determining changes in soil and bacterial numbers on surfaces over time. The detergent was the most important system component for controlling bacterial numbers, with the sanitizer contributing some control and the rinses very little. The numbers of *S. faecalis* on the surfaces were related to the amount of surface soil (detergent efficiency), and also to the inhibitory effect of solutions used before the intercycle rest period of 9.5 h between washing and the next soiling. *S. faecalis* did not grow during the intercycle period of 9.5 h at 30 C and a relative humidity of 80%, even on surfaces where a significant milk soil was present.

The sensory and toxicological quality of foods are often affected by microbiological contamination from equipment surfaces during storage, transport and manufacture. The hygienic condition of those equipment surfaces is controlled by the routine application of cleaning systems consisting of rinses, detergents and sanitizers. Despite the importance of these systems to the food industry, there is a serious lack of information on the factors which influence their effectiveness.

If the hygienic condition of food equipment is to be improved, the performance of the total cleaning system must be increased. This may sometimes be achieved by improving a single component of the system, such as the detergent (3,4) or the sanitizer (1). Greater benefits may be achieved by altering the structure of the total system (5). Manual studies of total system performance over multiple cycles are extremely laborious - and only one has been reported (5). Development of automated

cleaning simulators over the last 20 years has facilitated the examination of the total system (6,10,14), but these studies have concentrated on the control of soil accumulation and not included microbiological information.

Cleaning systems may be improved by being made simpler, cheaper and more effective. Eliminating the heating of detergents and rinses would result in economic savings. Reducing the numbers of cleaning solutions should lower costs and make it easier for operators to apply them reliably.

This simulator study was conducted to examine the chemical and microbiological performance of cold systems used to clean milk-soiled surfaces. Four variations of one system were used to examine the changes in performance over multiple soiling/washing cycles.

EXPERIMENTAL DESIGN

The most popular system for cleaning milking machines in Australia and New Zealand comprises a pre-milking iodophor sanitizer, a hot alkaline detergent and hot water sanitizing (11). In this experiment, variations of that system ("triple") were applied at lower temperatures and under soak cleaning conditions.

The components of each system are presented in Table 1. That shows that in the "triple" system (A.1) the milk soil was followed by a post-milk rinse of cold water, a warm alkaline detergent and then a post-wash rinse of cold water. The intercycle (rest) period of approximately 9.5 h commenced after the post-wash rinse and was followed by the pre-milk rinse of iodophor sanitizer, after which the milk soiling completed a 12-h soiling/washing cycle. System A.2 (no final rinse) was the same as A.1, except that the final water rinse was omitted. System A.3 (no post-milk rinse) was the same as 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" and was applied as a 3-g/L aqueous solution, and the iodophor "Klenz iodophor" (2% I_2 , 16% H_3PO_4) was applied at 1.5 ml/L (\equiv 25 mg available iodine/L). Both products were manufactured by Economics Laboratory (N.Z.) Ltd.

Each cleaning system was applied to one of the four test cells on the simulator described by Dunsmore et al. (8). The experimental systems were applied to the test pieces for 36 cycles of 12 h each with sampling done at cycles 1-2, 7-8, 17-18, 27-28 and 35-36. At each sampling cycle triplicate samples were taken for surface bacterial counts and gravimetric assessment of soil at the stages shown in Table 1.

MATERIALS AND METHODS

Preparation of test pieces

The test pieces ("slides") used measured 75 × 25 × 1 mm, and were

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made from Type 304 stainless steel with a 2B finish on both sides. They were numbered by a system of small marks on their edges. The slides were cleaned before use by scrubbing with alkaline detergent and brush in cold water, then rinsing in cold tap water, placing in a stainless steel washing box and boiling in 1% H₃PO₄ for 2 min, rinsing in 10 L of hot tap water, boiling in 1% NaOH for 2 min, rinsing twice in cold distilled water, boiling in distilled water for 2 min and drying. After placement into the test cells they were sterilized in an oven at 160 C for 2 h.

Preparation of cleaning solutions

All cleaning solutions were prepared with deionized water at the concentration at which they were to be applied to the test pieces. Calcium chloride and magnesium sulfate were added to give a total hardness of 200 mg/L as CaCO₃, with a Ca:Mg ratio of 3:2. The solution volumes applied were: pre-milk rinse, 2 L; milk, 2 L; post-milk rinse, 4 L; detergent, 4 L and final rinse, 2 L. The test cells were maintained at 30 C, except for the short periods when filled with a solution at another temperature. The numbers of "total" and *S. faecalis*-like organisms in the cleaning solutions were assessed by plating samples taken at the inlet to the test cells onto Tryptone Yeast Glucose Agar (TYGA) and TYGA containing 0.4 g of sodium azide/L (Azide Agar), respectively. Air in the test cells was maintained at 30 C and a relative humidity of 80%.

Preparation of inoculated milk

Milk used for soiling was whole milk obtained directly after pasteurizing and cooling from a dairy factory. The milk reservoir was filled, inoculated with the required organisms and then placed in the refrigerator. All soiling and cleaning solutions were replenished at least once every 48 h during a trial. The milk was inoculated with 10⁶ colony forming units (CFU)/ml of the *S. faecalis* (ATCC 8043) examined by Dunsmore et al. (8). The inoculum was prepared in the following manner: (a) A 500-ml conical flask, containing 100 ml of TYG broth was inoculated with the required culture. The flask was fitted with a glass side-arm to facilitate measurement of the optical density of the contents. (b) The broth culture was placed in a shaking incubator at 30 C until optical density reached 0.3 ± 0.03, which was equivalent to 5 × 10⁸ organisms/ml. (c) Seventy-two ml of the broth culture was placed in the 36-L of soiling milk to give a dose of 1 × 10⁶ CFU/ml of the organisms.

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 trial, re-weighing and determining the difference. A balance, accurate to ± 0.01 mg, located in a sealed box with constant temperature and humidity, was used for all weighings. A constant temperature of 25 C was achieved by a thermostatically-controlled infra-red lamp, and a constant relative humidity of 33% was maintained using 10 L of an agitated saturated solution of MgCl₂•6H₂O in the box (15). After pre-cleaning, slides used for gravimetric assessment were equilibrated in the balance box for 24 h, weighed and then placed in the test cells for experimental treatment. After removal from the test cells, they were stored in a desiccator containing silica gel until the experiment was completed. All slides were then placed in the balance box for 24 h and re-weighed.

Enumeration of viable bacteria on the surfaces of test pieces

The numbers of viable organisms present on the slides removed from the simulator were estimated by a swabbing/colony count technique. Two swabs which had been moistened in 10 ml of sanitizer inactivator solution (9) were each applied once, with a rotating motion, to all surfaces of the slides (the solution contained sodium thiosulfate 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 through a distance of 30 cm in 4 sec. Colony counts were made in duplicate on Azide Agar and also on TYGA. Plates were incubated for 48 h at 30 C before counting. Preliminary work showed recovery of organisms from the slides varied from > 85% for slide counts of 100, to > 95% for counts > 100,000 CFU. The log₁₀ (x + 1) transformation was applied to all mean colony counts before analysis.

RESULTS

The changes in the masses of the deposits which accumulated on the slide surfaces over 36 cycles are presented in Fig. 1a. System A.4 (no detergent) permitted the greatest accumulation of deposit, approximately six times that of the next treatment (A.2 - no final rinse). A.2 permitted twice as much deposit to accumulate as Systems A.3 (no post-milk rinse) and A.1 (triple), which

TABLE 1. Components of the 4 experimental systems and stages of sampling in detergent effect study.

Systems	System components					
	Pre-milk iodophor rinse (25 mg Av. l/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 stage	↑	↑	↑	↑	↑	↑
Surface bacterial count I ^a		S	M	R	D	F
Gravimetric ^b		G				

^aI - 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).

^bG - deposit weight after pre-milk rinse (immediately before milk soiling).

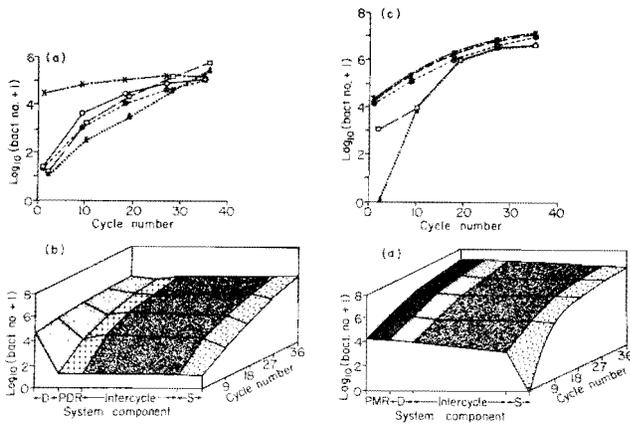


Figure 3. Changes in the numbers of viable *S. faecalis* organisms on stainless steel surfaces after components of treatment A.3 (no post-milk rinse) presented graphically (a) and three-dimensionally (b) and treatment A.4 (no detergent) presented graphically (c) and three-dimensionally (d). Key: After milk soiling —·X·—, After post-milk rinse —△—, After detergent —●—, After post-wash rinse —○—, After intercycle period —□—, After pre-milk sanitizer —△—, PMR, post-milk rinse; D, detergent; PDR, post-detergent rinse; Intercycle, intercycle period; S, pre-milk sanitizer.

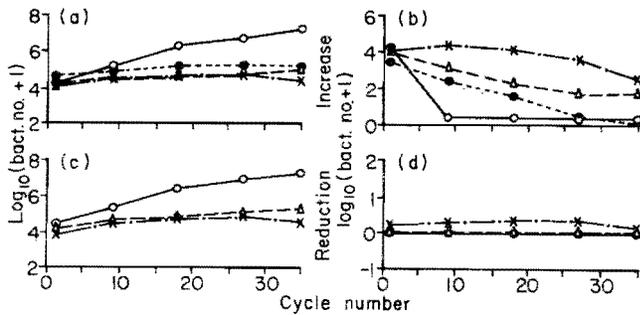


Figure 4. The influence of milk soiling and post-milk rinsing on the numbers of *S. faecalis* organisms on stainless steel surfaces with four cleaning systems: (a) Viable organisms after milk soiling, (b) Count increase with soiling, (c) Viable organisms after post-milk rinse, (d) Count decrease with post-milk rinse. Key: Treatment A.1 (triple) —·X·—, Treatment A.2 (no final rinse) —△—, Treatment A.3 (no post-milk rinse) —●—, Treatment A.4 (no detergent) —○—.

4b shows the numbers of organisms which adhered during the soiling operation at any time. Figures 4c, 5a, 5c, 6a and 6c show the numbers of survivors after exposure to the post-milk rinse, the detergent, the post-wash rinse, the intercycle time and the pre-milk sanitizer components of the system respectively, while Fig. 4d, 5b, 5d, 6b and 6d present the reduction in count caused by these respective components.

In terms of the mass of soil deposited, the systems were ranked A.1 and A.3 < A.2 < A.4 (Fig. 1a). In terms of the bacterial numbers surviving the total system, the systems were ranked A.1 < A.2 < A.3 < A.4 (Fig. 1b). Thus there was an overall relationship between the soil mass accumulated and the bacteriological control

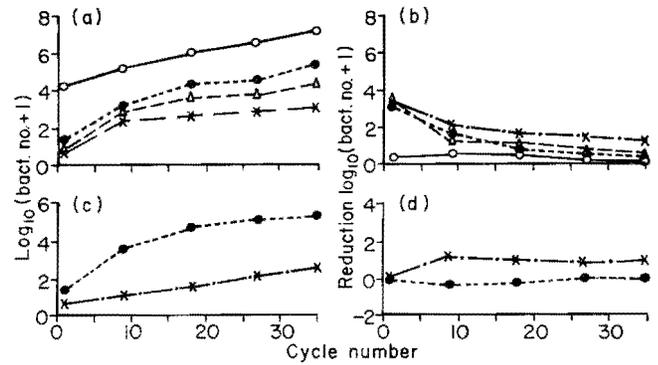


Figure 5. The influence of detergent washing and post-wash rinsing on the numbers of *S. faecalis* organisms on stainless steel surfaces with four cleaning systems: (a) Viable organisms after detergent washing, (b) Count decrease with detergent, (c) Viable organisms after post-wash rinse, (d) Count decrease with post-wash rinse. Key: Treatment A.1 (triple) —·X·—, Treatment A.2 (no final rinse) —△—, Treatment A.3 (no post-milk rinse) —●—, Treatment A.4 (no detergent) —○—.

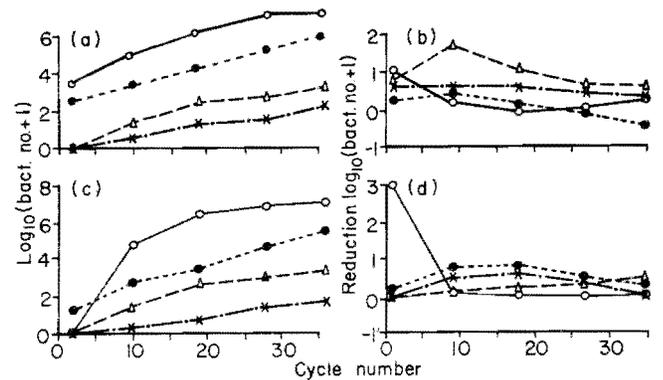


Figure 6. The influence of the intercycle period and pre-milk sanitizing on the numbers of *S. faecalis* organisms on stainless steel surfaces with four cleaning systems: (a) Viable organisms after intercycle period, (b) Count decrease in intercycle period, (c) Viable organisms after pre-milk sanitizing, (d) Count decrease with pre-milk sanitizing. Key: Treatment A.1 (triple) —·X·—, Treatment A.2 (no final rinse) —△—, Treatment A.3 (no post-milk rinse) —●—, Treatment A.4 (no detergent) —○—.

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.

In System A.1 (Fig. 2b), the post-milk rinse did not reduce the surface count. The detergent was the single most effective component of the system bacteriologically, but its effect diminished in later cycles. 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 *S. faecalis*. The sanitizer contributed very little to the total system performance. The system without a post-wash rinse (A.2 - Fig. 2d) showed a similar

pattern, except for a greater reduction in count during the intercycle period. In System A.3 (no post-milk rinse - Fig. 3b), 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 - Fig.3d), 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 were large numbers 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.

DISCUSSION

The simulator technique

The simulator used in this work is the first which has permitted detailed examination of the bacteriological performance of complete cleaning systems over time. It has the advantage of accurate control over the physical, chemical and biological conditions to which the test pieces were exposed. This greatly reduced the variability of results when compared to field evaluation of complete systems. It was sufficiently sensitive to permit detection of relatively small differences in soil mass or bacterial numbers within and between cycles, rather than just at the end of the experimental period, which until now has been the sole parameter used. Like other simulators, the cost involved in testing systems, in terms of cleaning materials and time, is only a small fraction of that required for field evaluation.

The simulator technique also has a number of disadvantages. Simulators are inherently complex, and the machine was one of the more complex built. As a result, the programming and control systems were highly sophisticated, which made them expensive and in need of frequent maintenance. Also, the relationship between the results obtained by both simulator and field assessment must be established before the results can be applied in the industry situation. Finally, the fact that the experimental situation is confined to only a few surface materials, a specific water quality, a limited range of organisms and assessment of only a few parameters, means that the results can be somewhat limited in their application. For example, on this simulator the test cells were filled and emptied slowly and the solutions were not agitated while in contact with the test pieces. 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. However, it limits application of the results to soak cleaning situations.

Despite their advantages and disadvantages, both the simulator and the field evaluation techniques have their importance in hygiene research and development. Any

radical new idea should be evaluated by field trial and, if found satisfactory, further development work can be quickly done by simulator 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.

Effect of system components

The numbers of *S. faecalis* organisms on the surfaces after soiling were most influenced by the amount of soil on the surfaces, increasing slowly as the cycle number increased (Fig. 4a); with System A.4 having far more than Systems A.3 A.2 and A.1. The numbers adhering at any time varied with the amount of soil on the surface (Fig. 4b). There was also a decrease in the numbers attaching during soiling over time, as the numbers on the surfaces before soiling increased, while the numbers after soiling changed little over time. The systems with poorest bacterial control (A.3, A.4) reached a stage in the later cycles where further attachment was possibly prevented by all sites being occupied.

The post-milk and post-wash rinses had negligible direct effects on the surface counts (Fig. 4d and 5d). As a result, the numbers of survivors after the post-milk rinse were the same as those after the milk soiling (Fig. 4c and 5c). Their indirect effects are discussed in the following paragraphs.

The reduction in bacterial count achieved by the detergent (Figure 5b) 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. (a) The importance of the efficiency of the detergent mixture used was demonstrated by the fact that the only difference between Systems A.2 and A.4 was the addition of detergent chemicals to the wash solution in System A.2 (b) The value of a post-milk rinse was shown by the fact that the detergent in System A.3 (which was not preceded by a post-milk rinse) was less efficient than that in System A.2 (where the detergent followed such a rinse). (c) Finally, the post-wash rinse was shown to make a significant contribution, as the use of a post-wash rinse in System A.1 improved the removal of the detergent/soil mixture remaining after the washing operation, permitting that system to perform more efficiently than System A.2 which lacked this post-wash rinse.

In all treatments, the intercycle conditions did not support significant growth of *S. faecalis* during the intercycle period (Fig. 6b). The reduction in count was greatest 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.

The same-pre-milking iodophor sanitizer was applied in all four systems. However, its effect varied with the

number of surface organisms at the start of the sanitizing operation and also with the amount of soil on the surfaces (Fig. 6b). The difference in the reduction in count between Systems A.3 and A.4 (Fig. 6b) which had similar surface numbers before pre-milk sanitizing (Fig. 6a) could result from the differing relationship between sanitizer and soil. The soil in System A.3 had been washed with detergent, while that in A.4 was merely rinsed with water - therefore they were qualitatively different.

The bacteriological control achieved by a system is the sum of the control achieved by its components. The modes of action of any component may include removal (detergency) and/or inhibition and/or killing. The efficiency of all the systems tested, as measured by the numbers of viable organisms on the surfaces at the time of soiling, diminished over time. This was due to accumulation of soil which remained after washing as the detergency process is never totally efficient.

The micro-ecology of the surfaces

Considerable information has been published on microbial populations on the surfaces of dairy equipment (5,12,13). That information shows that reasonably stable communities colonize the surfaces, the species represented in the colonies being influenced by many environmental factors.

Under conditions of this investigation the *S. faecalis* organisms did not grow on the surfaces at any time, and consequently could not be considered to be members of any active surface community. The organisms behaved as non-biological components of the milk soil, being deposited in constant numbers in the soiling milk, and removed by the detergent and rinses as part of the soil. This may not have occurred if the conditions for the intercycle period were altered, or if an alternative dosing organism was used.

Implications for the design of cleaning systems

The data show that the detergent was the most efficient system component, with smaller contributions made by the pre-soil sanitizer and rinses. This suggests that the system may be simplified by eliminating the post-soil rinse; Clegg and Cousins (2) have shown that the contribution of this rinse to deposit control may be either positive or negative, depending on the detergent employed. The limited efficiency of the pre-soil sanitizer indicates that the sanitizer may be more efficient if applied as the post-detergent solution, which would ensure a residual effect of anti-bacterial reagents on the

soil during the intercycle period. The implications of this change are investigated in a comparison paper (7).

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