Bacteriological Control of Food Equipment Surfaces by Cleaning Systems. II. Sanitizer 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 30 12-h soiling and washing cycles with four cleaning systems. The soiling milk was inoculated with $5 \times 10^5$ Streptococcus faecalis and $5 \times 10^5$ Enterobacter aerogenes organisms/ml. One system included a pre-milk rinse of water (20 C), soiling milk (30 C), wash with alkaline detergent (50 C) and two rinses with water (20 C). In the second system, an iodophor sanitizer (25 mg available iodine/L, 20 C) was substituted for the pre-milk water rinse, and in the third the iodophor was substituted for the final water rinse. In the fourth system, the iodophor was applied instead of the final water rinse, but was left for the 9.5-h intercycle period and drained immediately before the next milk soiling. Results showed that the sanitizer can be an important and, under some conditions, the most effective system component in controlling the bacterial population on surfaces. Of the short contact-time sanitizer applications, the post-wash sanitizer was more effective in reducing the bacterial count than the pre-milk sanitizer, but application of the sanitizer for the complete intercycle period completely eliminated all viable bacteria and was thus far more effective than either of the short contact-time methods. It was also shown that the sanitizer can play an important role in the detergency of the system. The organisms studied (S. faecalis and E. aerogenes) differed in their soiling characteristics and survival in intercycle conditions.

Foods are often contaminated microbiologically from surfaces of equipment in which they are harvested, stored, transported and processed. The quality of food products can be improved by reducing the amount of contamination which occurs. The major means of achieving this reduction is to increase the performance of the detergents and sanitizers in the cleaning systems which are applied routinely after each use of the equipment.

Sanitizers are traditionally applied after the detergent, but in equipment such as milking machines they may be applied either 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 field studies on pipeline milking machines (7), but may require a following water rinse to prevent sanitizer residues from contaminating the milk (3).

This sanitizing is, however, the only cleaning task which is necessary before milking and is therefore an operation which many dairymen find onerous. This can be overcome by applying the sanitizer immediately after the detergent, where it is merely one component of the total post-milk washing program. This can also have the further advantage of reducing any sanitizer residue problems as the long intercycle time may overcome evaporation and drainage the need to rinse the sanitizer from the surfaces before the next milking.

Because the efficiency of both detergency and sanitizing are markedly improved by increasing temperature, cleaning milking machines with systems comprising only cold solutions places limits upon the performance of the system. The possibility existed that increasing the contact-time of cold detergents and sanitizers could overcome some of the disadvantages of this cold system. With milking machines, the intercycle period offers the opportunity to have a solution contact the surface for 10 h, which changes the microenvironment of any organisms on the surface for that period considerably.

This is the second paper in a series which examines the contribution of system components to performance of the total cleaning system. The first (7) investigated the effect of the major detergent in the system. The aims of this study were to investigate the contribution of the sanitizer to performance of the total system, the relative performance of pre-milk and post-wash sanitizers, and also the bacteriological control achieved by a sanitizer applied throughout the complete intercycle period.

EXPERIMENTAL

Four experimental cleaning systems were applied to test pieces over multiple soiling/washing cycles. Details of the four treatments investigated are presented in Table 1. System B.1 (no sanitizer) consisted of four steps; a pre-milk rinse of cold water, soiling milk, 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 test pieces until drained away immediately before the next milk soiling, before

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which no pre-milk solution was applied. The alkaline detergent used was “Alka-Kienz” (3 g/L), and the iodophor was “Klenz-Iodophor” (2% I₂, 16% H₃PO₄, at 1.5 mg/L). Both products were supplied by Economics Laboratory (N.Z.) Ltd.

Each of the four experimental treatments was applied for 28 cycles to a test cell of the simulator described by Dunsmore et al. (5). The stainless steel test pieces (“slides”) used and slide preparation method were as described by Dunsmore (1). Sampling was done at cycles 3-4, 9-10, 15-16, 21-22 and 27-28. At each sampling, quadruplicate slides were taken for enumeration of surface bacteria and gravimetric assessment of the quantity of soil by the methods described by Dunsmore (1) at the stages shown in Table 1.

The inoculated soiling milk was prepared by the method of Dunsmore (1), with the following modifications. The milk was inoculated with 36 ml of broth cultures of each of Streptococcus faecalis (ATCC 8043) and Enterobacter aerogenes (NCIB 418) to achieve $5 \times 10^5$ of each in the milk. The air in the test cells was maintained at 30°C and a relative humidity of 85%.

The cleaning solutions were also prepared by the method described by Dunsmore (1). Aerobic plate counts on the cleaning solutions entering the test cells showed the iodophor solution and alkaline detergent to contain <1 CFU/ml, but the water rinses were contaminated with an organism resembling E. aerogenes due to malfunction of the filter system on the water supply.

The masses of soil and the numbers of viable bacteria on the slides were determined by the methods described by Dunsmore (1). Tryptone Yeast Glucose Agar (TYGA) was used for “total counts,” Azide Agar (TYGA with 0.4 g of sodium azide/L) was used to enumerate S. faecalis, and Violet Red Bile Agar (VRBA) for E. aerogenes.

**RESULTS**

Changes in soil mass on the slide surfaces with the four treatments are presented in Fig. 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 small initial increase to a plateau level, 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

**TABLE 1. Cleaning systems applied and sampling stages.**

<table>
<thead>
<tr>
<th>Cleaning system</th>
<th>Components of cleaning systems</th>
<th>Pre-milk</th>
<th>Milk</th>
<th>Alk. detgt. 50°C</th>
<th>Water rinse 20°C</th>
<th>Final soln</th>
<th>Intercycle</th>
</tr>
</thead>
<tbody>
<tr>
<td>B.1 No sanitizer</td>
<td></td>
<td>Water</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>Water</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td></td>
<td>20 C</td>
<td></td>
<td></td>
<td></td>
<td>20 C</td>
<td></td>
</tr>
<tr>
<td>B.2 Pre-milk sanitizer</td>
<td></td>
<td>Iodophor</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>Water</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td></td>
<td>25 mg Av.</td>
<td></td>
<td></td>
<td></td>
<td>20 C</td>
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<td></td>
<td></td>
<td>1/L, 20 C</td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>B.3 Post-wash sanitizer</td>
<td></td>
<td>Water</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>Iodophor</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td></td>
<td>20 C</td>
<td></td>
<td></td>
<td></td>
<td>25 mg Av. 1/L</td>
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<td></td>
<td></td>
<td></td>
<td>20 C</td>
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<tr>
<td>B.4 Intercycle sanitizer</td>
<td></td>
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<td></td>
<td></td>
<td>Iodophor</td>
<td>+</td>
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<td>25 mg Av. 1/L</td>
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<td></td>
<td>20 C</td>
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</tr>
</tbody>
</table>

**Sampling stages**

- 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 pre-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).
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 after cycle 20, compared to the no-sanitizer treatment (System B.1).

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 2a, 3a and 3c. Similar data for *E. aerogenes* are presented in Figures 2b, 2d, 3b and 3d. In these illustrations the count at the start of the detergent and post-detergent rinse is the same as that after milk soiling. Similarly the count after the pre-milk solution (Systems B.1, B.2 and B.3) or intercycle period (System B.4) is the number on the surface immediately before a soiling. Thus count variation traversing from left to right at any one cycle number show the change in count achieved by the components of the cleaning system. Traversing in the other plane provides information on the way the influence of any one system component varies as the cycle number is increased.

System B.1 (no sanitizer) had similar effects on numbers of *S. faecalis* (Fig. 2a) and *E. aerogenes* (Fig. 2b). The detergent and rinse reduced the count significantly after the milk soiling, but the magnitude of the effect diminished with increasing cycle numbers. 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. During the intercycle period the decrease in numbers of *E. aerogenes* was much greater than that of *S. faecalis* in the early cycles, but again there was little change at the end of the experimental period. The pre-milk rinse had little effect on *S. faecalis* but the number of *E. aerogenes* showed a significant increase due to contaminated rinse water.

The detergent was again the most effective component of System B.2 (pre-milk sanitizer, Figures 2c and 2d). The final solution of water had a negligible effect with *S. faecalis*, but the count increased due to contamination with *E. aerogenes*. There was a small decrease in numbers during the intercycle period at all cycle numbers. The iodophor sanitizer applied before milking slightly reduced the numbers of *S. faecalis* in early cycles, but had no effect in later cycles. The sanitizer reduced the numbers of *E. aerogenes* at all cycles.

The detergent also greatly reduced the surface counts of *S. faecalis* (Fig. 3a) and *E. aerogenes* (3b) in System B.3 (post-wash sanitizer). However, in this system application of the iodophor in the post-wash step of the system 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

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**Figure 2.** Changes in the numbers of viable bacteria on stainless steel surfaces after components of two cleaning systems presented three-dimensionally. (a) System B.1 (no sanitizer) *S. faecalis*, (b) System B.1 (no sanitizer) *E. aerogenes*, (c) System B.2 (pre-milk sanitizer) *S. faecalis*, (d) System B.2 (pre-milk sanitizer) *E. aerogenes*. Key: D + R - Detergent and rinse, FS - Final solution, Intercycle - Intercycle period, PMS - Pre-milk solution.
rinse water.

In System B.4 (intercycle sanitizer), the detergent rinse had a similar effect on the counts of *S. faecalis* (Fig. 3c) and *E. aerogenes* (Fig. 3d) as in the other systems. In this system, however, application of the sanitizer for the whole of the intercycle period made it the most effective bacterial control agent in the system. The control of both *S. faecalis* and *E. aerogenes* was complete, no viable organisms of either species remaining at the time of the milk soiling (after drainage of the intercycle sanitizer) for the whole experimental period of 28 cycles.

The influence of system components upon numbers of *S. faecalis* on slide surfaces, in terms of both survivors and reduction in count, is shown in Figures 4a, 4b (milk soiling), 4c, 4d (detergent and rinse), 5a, 5b (final solution), 5c, 5d (intercycle time) and 5e, 5f (pre-milk solution). The equivalent data for *E. aerogenes* are not presented due to lack of space. The effect on the numbers of *S. faecalis* and *E. aerogenes* of the three application methods of the iodophor sanitizer solution are presented in Fig. 6. Similar data for the four total systems are presented in Fig. 7.

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 Fig. 8. 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). In early cycles *S. faecalis* dominated the microflora, while in later cycles, *E. aerogenes* 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.
Figure 5. The influence of the final solution and intercycle period on the numbers of S. faecalis organisms on stainless steel surfaces with four cleaning systems. (a) Viable organisms after final solution, (b) Count decrease with final solution, (c) Viable organisms after intercycle period, (d) Count decrease with intercycle period, (e) Viable organisms after pre-milk solution, (f) Count decrease 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) — .

Figure 6. The influence of the timing of sanitizing on the numbers of organisms on stainless steel surfaces. (a) Numbers of viable S. faecalis after sanitizing, (b) Decrease in count of S. faecalis after sanitizing, (c) Number of viable E. aerogenes after sanitizing, (d) Decrease in count of E. aerogenes after sanitizing. Key: System B.2 (pre-milk sanitizer) — , System B.3 (post-wash sanitizer) — , System B.4 (intercycle sanitizer) — .

Discussion

Total system performance

The soil mass data (Fig. 1) show that addition of an iodophor improved the detergency of the system by reducing soil accumulation. This effect was demonstrated by Scott et al. (8), and results from the acid and surfactants in the iodophor enabling it to act as a mild acidic detergent. The influence of the iodophor varied with its timing within the system. The similarity in soil accumulation characteristics between those systems in which the iodophor was the final pre-milk solution suggested that residues of the iodophor on substrate surfaces affected 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 (Fig. 1). In terms of the numbers of bacteria surviving at the end of the experimental period, the systems were ranked B.4 < B.3, B.2 < B.1 (Fig. 7a and 7c). Thus the bacteriological performance of the system was not related to its detergency, but in fact was related to the relative efficiency of the sanitizer used in the system (Fig. 6a and 6c). This suggests that the amounts of soil on the surfaces in this experiment could have been insufficient to limit the bactericidal efficacy of the sanitizer. Alternatively, as there was no growth of the organisms within the soil, the performance of the sanitizer may merely reflect the ability of the sanitizer to
act on the immediate surface layer - at each new cycle soil and organisms would be deposited, and the sanitizer may have merely affected that thin surface layer - a layer shallow enough not to influence performance of the sanitizer. For example, at each cycle with the intercycle sanitizer (System B.4) a thin layer of sterile soil would be added to the soil remaining from previous cycles.

Performance of system components

After milk soiling, the numbers of S. faecalis organisms on the surfaces increased slowly with time, with minor differences between the treatments (Fig. 4a). The numbers attaching at any time (4b) were influenced by the amount of soil on the surface and the numbers on the surface after the premilk treatment.

The detergent and rinse components were common to all treatments, so any differences were due to the different components of these systems. The efficiency of the detergent, as shown by the reduction in count (Fig. 4d) was inversely related to the amount of soil on the surface (Fig. 1). This was shown in both the relative performance of the detergent between systems and reduction in detergent efficiency with increasing cycle number.

The final solutions in Systems B.1 and B.2 were both water rinses, and consequently had negligible effects upon the surface count (Fig. 5b). Application of a post-wash sanitizer in System B.3 significantly reduced numbers of organisms in early cycles when the soil mass was low, but this effect diminished with time. Application of the intercycle sanitizer in System B.4 was totally effective in controlling bacteria at all cycle numbers.

Numbers of S. faecalis were reduced during the intercycle period (Fig. 5c). This reduction was influenced by two factors; the amount of soil on the surface and application of a sanitizer as the final treatment before the intercycle period (System B.3).

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 was related to both the overall efficiency of the system (cf systems B.1 and B.3 in Fig. 5a) and use of a pre-milk sanitizer.

The iodophor sanitizer was applied in three ways in the different 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 6b and 6d show that those treatments were clearly different in their relative efficiencies. The long exposure time of the intercycle sanitizer enabled it to eliminate all viable bacteria. The apparent increase in the efficiency of this sanitizer over time (in terms of the reduction in bacterial numbers - Fig. 6b and 6d) merely reflects the increasing numbers of organisms exposed to the sanitizer after the washing solutions had been drained. It is possible that if the experiment had been continued for the longer periods which usually occur in practice, 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 in the soil.

Of the two systems where the iodophor was applied as a short contact-time rinse, reductions in counts show that the post-wash application (System B.3 - Fig. 6b, 6d) was more effective in the early cycles than the pre-milk application (System B.2). The post-wash application may not give the bacteria an opportunity to recover from the stress caused by the detergent, resulting in a more pronounced bactericidal effect. The pre-milk application gave the bacteria a 9.5-h period to recover from the washing solution, which may have permitted them to survive the sanitizer a little more efficiently.

Micro-ecology of equipment surfaces

Both of the inoculated organisms attached to 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 (Fig. 8a). However, E. aerogenes attached in relatively small numbers in early cycles, but increased in numbers similar to S. faecalis after 28 cycles. A similar relationship was demonstrated with these organisms in their attachment to glass in a skim milk soil by Dunsmore and Bates (2).

As in the experiment described by Dunsmore (1), there was no occasion during which the conditions were suitable for growth during the intercycle period, although there were situations when there was no reduction in count - particularly where a large amount of soil had accumulated. This probably resulted because the relative humidity of 85% was still insufficient for growth of the test organisms used. The organisms differed in their ability to survive the intercycle period - E. aerogenes survived the intercycle period poorly in early cycles when there was little soil, but much more effectively in later cycle numbers (Fig. 8d).

Implications for cleaning system design

The most efficient application of a sanitizer is as an intercycle solution. This was shown in this simulator study to be the most effective bactericidally, even where the amount of surface soil was high. In this application, the sanitizer had the dual benefits of long contact time with the surface, and provision of inhibitory conditions for the complete intercycle period. Also, as the sanitizer is only drained immediately before the product passes through there is no opportunity for recontamination of the equipment surfaces. Maxcy and Shahani (6) showed that the long contact time of the intercycle sanitizer application permitted the concentration of the sanitizer to be reduced, which would save on costs and also reduce the possibility of sanitizer residues entering the food. In practice, this type of intercycle sanitizer is limited to small-volume equipment such as pipe-lines, where an economical volume of sanitizer could be applied. It would not be appropriate for large-volume equipment like processing vats and storage vessels.

In this simulator study, the post-detergent application

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of the sanitizer was more effective than the pre-milk application, which is contrary to the results which Middleton et al. (7) obtained in a field trial on milking machines. One possible reason for this difference may be that the double stress of the alkali-acid sequence of the alkaline detergent/iodophor double stress used in this work may be more effective than the alkali-alkali sequence of the alkaline detergent/hypochlorite used in the field study. A more likely explanation is that the pre-milking application of the sanitizer in the field reduced the re-contamination of the surfaces which occurs in the intercycle period (conditions were controlled in the simulator so re-contamination could not occur).

The pre-milk application of the sanitizer raises the possibility of sanitizer residues contaminating the milk (3). This can be overcome by rinsing the sanitizer from the equipment with water, which complicates the system and also raises the problem of re-contamination if the quality of the water is poor. The very significant effect that contaminated rinse water can have is demonstrated in Fig. 3b. The value of the complementary application of alkaline and acidic detergents is studied in the third paper in this series (4).

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