

INTERACTION OF CHEMICAL, THERMAL AND PHYSICAL ACTIONS ON THE
REMOVAL OF BACTERIA FROM MILK CONTACT SURFACES

by

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Summary:

The effects of chemical, thermal and physical actions on the removal of bacteria from milk contact surfaces were investigated. Tests were performed over the range of flow and temperature conditions normally encountered in air injected milking CIP systems. Significant differences were observed between shear, temperature and different chemicals. Interactive effects between detergent and shear and detergent and temperature shear were noted. The test procedure developed proved to be a reliable and repeatable method of measuring bacterial removal from milk contact surfaces.

Keywords:

Milking Systems, Sanitation,

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Background and Literature Review

Cleaning and disinfection of food contact surfaces are accomplished by a combination of physical, thermal and chemical processes. The circulation of sufficient volume of cleaning solutions at sufficient velocity and temperature is required to adequately clean milk contact surfaces. Failure of Clean In Place (CIP) systems often results from inadequate velocity or contact time of the cleaning solution. A small amount of residual soil may facilitate bacterial attachment, survival and growth. If not inactivated or removed during cleaning, remaining bacteria may eventually detach and contaminate the milk supply. This may affect the quality, and if pathogens are present, safety of milk.

Current recommendation for air injected cleaning regimes have been developed largely on a trial and error basis. As milking installations become larger and more complex with the addition of milking automation the cleaning task becomes more difficult. Cleaning problems are generally detected by elevated bacterial plate counts of the product after many soiling/cleaning cycles. When this occurs, bacterial contamination is likely to have already affected a large volume of product. This also results in a loss of milk quality premiums paid to the dairy producer.

Milking system pipelines are subject to special requirements which distinguish them from those in dairy and other food processing plants. Milking system pipelines are considerably larger in diameter than product lines in dairy plants because they must carry both milk and air in a stratified flow condition during the milking process. Most pipeline systems in dairy and food processing plants are cleaned by flooding the lines and circulating cleaning solutions under pressure with a centrifugal pump. The amount of hot water and detergents required to flood pipelines increases proportionally with the square of the pipeline diameter.

Milking equipment is commonly cleaned using air injected CIP systems producing 'slug' flow in the milkline. The flow dynamics are very different from those encountered in food plant situations. Air injected CIP systems make use of the milking vacuum pump to supply the forces required to circulate cleaning solutions thus eliminating the need for a large circulation pump. Air is injected to increase the circulating velocity and mechanical cleaning action of the wash solution. The objective in air injected flow is to form a 'slug' of cleaning solution and move this slug around the system to provide adequate turbulence and contact time on all surfaces to perform the cleaning and sanitizing functions. Slug flow is characterized by the passage of discrete liquid slugs. The slugs may vary from a few centimeters up to several meters in length. The slugs move with high velocity and fill the entire pipe cross section. The area between the slugs contains a slower moving liquid film in the bottom of the pipe.

The slug velocities developed (18 to 44 ft/s, 6 - 12 m/s) are 5 to 10 times higher than those encountered in continuous flow CIP circuits. The wall shear stress developed are ten to thirty times higher than those developed in continuous flow CIP systems. The contact time between the slug and pipe wall is, however, quite short. A 2 meter slug moving at 10 m/s will have a contact time of 0.2 seconds. Air injection reduces the water requirements for cleaning when compared

to fully flooded lines. Previous work has been oriented toward much lower shear stress found in flooded systems, typical of cleaning systems found in food and dairy processing plants.

Previous work on flooded systems has shown that even with acceptable flooded CIP systems in milking and dairy environments, microorganisms can remain on equipment surfaces. Maxcy (17) detected bacteria from both the raw and pasteurized sides of cleaned equipment in a dairy plant. Mattila (15) isolated a variety of organisms from a milking line which had been cleaned by two CIP systems. Three independent surveys (2,7,19) indicated that *Listeria* spp. could be isolated from milk processing and dairy plants. Frank, Gillett and Ware (7), examined and found the presence of other microbial groups in addition to *Listeria* spp., in dairy plants. Czechowski (5) found that bacteria remained on Buna-n rubber gaskets in milk processing equipment following a CIP operation. The number of bacteria increased with the length of time the gaskets were in use. In simulated CIP systems, Dunsmore et al.(6) and Maxcy (17) observed residual microorganisms on cleaned surfaces. In addition, Maxcy noted that the microflora of the cleaned model equipment was similar to that of the pasteurized, packaged milk. Stone and Zottola (25) demonstrated with a stainless steel milk pipeline model system that CIP treatment inactivated *Pseudomonas fragi*, but the organisms remained attached to the stainless steel.

Bacteria exposed to inert surfaces can attach readily. Under suitable conditions, organisms that remain irreversibly attached grow and develop into micro-colonies, and with time, extensive biofilms may be formed (1,4). Portions of the biofilm may detach and be transported to other parts of the system. Detachment may occur due to shear forces. Powell and Slater (20) showed that the rate of removal of *Bacillus cereus* cells from glass surfaces increases with increasing shear stress. The biofilm cells excrete extracellular polymeric substances (EPS), forming a matrix (often referred to as glycocalyx) which helps to stabilize the biofilm. The precise chemistry of the matrix is usually not known. In a few instances where EPS has been studied in detail, polysaccharides were found to be present (3). Other substances, such as proteins, nucleic acids, and components from the liquid phase, may also be present, and affect the properties of the biofilm. Bacterial attachment, EPS production and biofilm formation are affected by a variety of factors including the organism, attachment surface, nutrient availability, and temperature. Cells in biofilms are generally hardier than their planktonic counterparts, and exhibit increased resistance to antibiotics, disinfectants, and heat (4,8,12,13,14,23). It is speculated that the EPS plays a role in conferring resistance, but the mechanism is not known.

Previous work has shown that bacteria can attach and form biofilms on dairy equipment surfaces (8,11,12,23). The flow conditions encountered in air injected milking equipment CIP systems have been studied (21,22). No comparable work has been done on the effects of the high shear encountered in air injected milking systems on the removal of bacteria from milk contact surfaces and the interaction between high shear and chemical and thermal actions.

The objectives of this study were to: 1) Develop a method to test the effectiveness of removing bacteria from milk contact surfaces, 2) Characterize the effects of the high level of shear encountered in air injected CIP systems on bacterial removal, and 3) Investigate the interactive effects of physical, chemical and thermal actions on bacterial removal.

Materials and Methods

Four sections of 2 ft x 3 in (60 cm x 7.5 cm) stainless steel pipe (type 304, #4 finish) were constructed, each with four removable collars. Each collar is fitted with five removable stainless steel test chips (ca. 0.2 cm² diameter) placed at five locations around the perimeter of the collar. When in place, the inner surface of each chip is level with the inner surface of the test section. This is important because any protrusion or indentation from the rest of the pipe will interfere with the flow dynamics of the cleaning fluid, and modify the shear stress created along the pipe surface. Previous studies have used test chips protruding for the pipe wall or suspended in the flow away from the pipe wall. Even small physical disruptions can cause significant changes in the flow pattern and hence the effective shear stress experienced by the test piece. This method maintains the micro flow structure in the test section.

Prior to each experiment, the pipe sections were cleaned with a warm alkaline detergent solution, rinsed thoroughly with double distilled water, and allowed to air dry. The ends were sealed and the tubes autoclaved. Each test section was mounted horizontally on a shaft/rotor apparatus, filled with 100 ml of a 1:1,000 dilution of pasteurized whole milk inoculated with about 10⁹ cfu/ml of a *Lactobacillus* culture. The culture was originally isolated from raw milk. The test section was rotated at 10-12 rpm for two hours at room temperature to allow uniform deposition of milk soil and lactobacilli on the surface of the pipe.

The milk was removed from the pipe section, which was then placed in the milking system at a predetermined location and subjected to specified cleaning conditions. Test chips from one collar were removed prior to cleaning and processed for enumeration of lactobacilli immediately. These served as the controls. The surface of each removed test chip is swabbed with an alginate swab. The tip of the swab was broken off in a 0.1% peptone solution, and appropriate dilutions plated on APT agar plates with bromocresol purple and incubated for 4 days at 25°C. The swabbed chips were then placed back in their respective positions in the collars. The collar was replaced in the pipe section and the cleaning cycle started. The chips removed after cleaning will be treated similarly. For each experiment, three collars were used for viable count. For some runs one of these three collars was used for scanning electron microscopy (SEM).

Chips used for SEM were processed using two different methods. In the first phase of the research a fixation method employing ruthenium red was used. The chips were placed in a solution containing 0.15% ruthenium red in 0.1M cacodylate buffer for two hours. They were then transferred to a second solution containing 0.05% ruthenium red in buffer with 2% glutaraldehyde, for another two hours. The chips were then dehydrated in an ethanol series of 25, 35, 50, 75, 85, 95, and 100% for ten minutes each. This procedure produced poor clarity of SEM images. Another method was used in the second phase of the research, described by Krysinski (12). Chips were placed in a 0.1mg/ml concanavalin A for twenty minutes, then transferred to a 0.1M phosphate buffered saline (PBS) solution containing 0.1% CaCl₂ and 0.1% MgCl₂ for another twenty minutes. The chips were then fixed in a 0.2 M cacodylate buffer with 1% glutaraldehyde overnight at 4°C. The same step-wise dehydration series was used in this method as previously. This method improved the clarity of SEM images.

A series of tests were run using plain water at room temperature to isolate the effects of physical

action on bacterial removal. This series used a full size milking system with 73 meters of 3 inch (7.5 cm) pipeline. Test sections were mounted at two locations; one near the beginning of the slug travel (32 ft, 10 m) and one near the end of slug travel (207 ft, 63 m). Wall shear stress ranging from 50 to 250 N/m², were examined. This covers the range commonly encountered in air injected systems. Each test was performed using 18 air injection cycles producing 15 slugs at the test section.

A second series was run using plain water and several formulations of alkaline detergents at temperatures ranging from 43°C to 70°C and at two levels of shear. Two replicates were performed for most test conditions. This series used a reduced size milking system with 17 meters of 3 inch (7.5 cm) pipeline. The test section was mounted 10 meters from the start of slug travel. The levels of shear were more controllable in this smaller system than in the larger system used in previous trials. The number of slugs was reduced to 5 because of the higher level of bacterial removal achieved with elevated temperature and cleaning chemicals than in previous tests with water at room temperature. The SAS general linear models procedure was used to analyze the data (24).

Results and Discussion

Effect of Shear: The effects of shear using plain water at room temperature and 15 slugs are illustrated in Figure 1. Increased air admission improved cell removal up to a point after which cell removal was reduced with increasing air admission. The same trend was apparent at both test locations. The reduction in cleaning action at the highest level of air admission was more pronounced at the 63 m location. This is because the slug length has been reduced and air entrainment was increased due to excessive air admission. This corresponds with results obtained previously using other methods of assessing mechanical cleaning action (21).

The effect of shear in the hot water and detergent trials is illustrated in Figure 2. Two levels of shear, representing the range recommended from previous trials, were investigated in more controlled conditions. Higher cell removal was achieved at 138 N/m² than at 56 N/m². The degree of repeatability and level of significance were improved with greater control over the test conditions using the shorter 12 meter pipeline.

The relationship between cells remaining and radial location of the test chip using plain water at room temperature and 15 slugs is shown in Figure 3. Greater removal was achieved in the upper portion of the pipe. This indicates that the standing liquid layer reduces the local velocity and wall shear in the bottom of the pipe. The relationship between cells remaining and radial location of the test chip using hot water and 5 slugs is shown in Figure 4. The difference between chip locations is reduced. The relationship between cells remaining and radial location of the test chip using hot detergent and 5 slugs is shown in Figure 5. The difference between chip locations was no longer significant. This trend is probably due to the increased importance of thermal and chemical action relative to physical action on cell removal and destruction.

Effect of Temperature: The effect of temperature on cell removal is illustrated in Figure 6. There was no significant difference in cell removal at the lower three temperature ranges (110 - 130 F, 43 - 54 C). Increasing cell removal was observed as the temperature

increased above 130 F (54 C). This effect was expected because the bacteria used for testing were shown in static tests to withstand temperatures up to 130 F (54 C) with no significant reduction in cell numbers. Reduction in cell numbers was observed for temperatures 140 F (60 C) and above.

Effect of Detergent: The effect of the different detergents tested is illustrated in Figure 7. There was a significant difference observed between plain water and all detergents tested. Detergent A was also significantly different than Detergents B, C and D. There were some interactive effects between detergent and temperature. Detergent A did not differ from plain water under low temperature conditions (Figure 8). Detergent B performed better than A, C and D under conditions of low shear (Figure 9). Detergents C and D performed better than A and B under conditions of high shear (Figure 10).

Summary

The effects of interaction of chemical, thermal and physical actions on the removal of bacteria from milk contact surfaces were investigated. Tests were performed over the range of flow and temperature conditions normally encountered in air injected milking CIP systems. Significant differences were observed between shear, temperature and different chemicals. Interactive effects between detergent and shear and detergent and temperature shear were noted. The test procedure developed proved to be a reliable and method of measuring bacterial removal from milk contact surfaces.

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