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Quantitation of Proteinaceous Soil Removal From Clean-in-Place Systems¹

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ABSTRACT

The protein of milk residue can be quantitated to evaluate a cleaning process by monitoring the accumulation of protein in a test-wash solution. The test for protein is based on precipitation of hard water cations with orthophosphate and flocculation with a coagulant for rapid sedimentation of the protein. The sediment is collected on filter paper and stained with xylene brilliant cyanin G. Color reaction on the filter paper is proportional to protein content of the original test solution and is estimated visually. For more precise quantitation, the stain is released into a solution for optical measurements. The lower limit of sensitivity is approximately 34 ng of protein per ml in the original caustic test wash. Quantity of added protein to a caustic test wash and optical density of the final test dye solution showed a correlation coefficient of .99. The method developed in this work should be useful in studying clean-in-place regimen to evaluate commercial practices and fundamentals of the processes.

INTRODUCTION

Evaluation of cleaning processes poses a challenge for the modern complex dairy industry (1, 5, 8). Even microquantities of soil may support growth of microorganisms (5). Research on soil deposition and removal processes with the associated methods of evaluation have been reviewed by Kulkarni et al. (4).

To be useful in the modern dairy industry, a method for evaluating effectiveness of cleaning must be capable of detecting soil in nanograms

for a piece of equipment or micrograms for a system as a whole (6). The most successful approach has been analysis of cleaning solutions to quantitate soil removal from the equipment (2). However, quantitation of such an important constituent as protein in a cleaning solution is not sufficiently sensitive with present methodology to evaluate the final phases of the cleaning process.

A highly sensitive and practical method for monitoring cleaning processes would contribute to improved equipment and process design, improved cleaning materials, and minimization of waste. This work was directed to concentration of soil in cleaning solutions and application of the most sensitive available methods for protein determination.

MATERIALS AND METHODS

Protein Concentration

Because of low quantities of protein in a large volume, a need for concentration was recognized. Various systems such as drying, condensing, and precipitating were evaluated. The only apparent workable system was based on utilizing the highly insoluble nature of cations of hard water in combination with orthophosphate. Flocculation with a coagulant used in water purification incorporated the proteins into the precipitate and thereby aided sedimentation. This system provided a relatively rapid and effective technique for concentrating the protein. The final detailed procedure follows.

The test-wash solution contained hard water constituents, .9% NaOH, and .1% Na₃PO₄. This solution was acidified to pH 9.5 with 12 N HCl. A one-liter sample was agitated rapidly with a magnetic stirrer while approximately 2 mg of Calgon Coagulate Aid 243 were added. Rapid agitation was continued for 2 min after which the rotational velocity was slowed to 30 rpm. After a few minutes the sample was removed from the stirrer. The floccule completely

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settled within 15 min. Most of the clear supernatant was removed with a gentle syphon. The remaining materials were transferred to centrifuge tubes for treatment at $670 \times g$ for 5 min. An approximate 4-ml pellet was formed from which the supernatant was decanted. The insoluble salts of hard water and orthophosphate were dissolved and the concentrated protein was coagulated by adding .5 ml of 12 N HCl. The resulting sample suspension was filtered with a Buchner funnel containing a 1.5 cm disc of Whatman No. 42 filter paper. The disc on which the protein was collected was dried 30 min at 60 C.

Protein Detection and Quantitation

Protein detection systems which were evaluated included fluorescamine analysis, the Lowry test, and the Bramhall method (3). Only the Bramhall method, with certain adaptations described below, was suitable.

The filter disc, with the dried protein as indicated above, was placed in a cold 7.5% trichloroacetic acid solution, which then was heated to 80 C and maintained at that temperature for 30 min. The disc was rinsed in a 1:1 mixture of ethyl ether and ethanol, followed by rinsing with ethyl ether. After drying, the discs were stained for 15 min at 50 C with xylene brilliant cyanin G (Microme No. 1224), which was 10 mg/ml in a 7% acetic acid solution. Excess dye was removed from the disc by soaking 5 min each in six consecutive solutions of 7% acetic acid at 55 C. Color intensity of the resulting disk was proportional to protein concentration in the original test-wash solution. Refinement in quantitation was achieved by destaining the disc in 5 ml of methanol, water, and ammonia (66:34:1). Dye released into this destain was measured colorimetrically at 610 nm.

Laboratory Pasteurizer Soiling, and CIP System

A laboratory model Alfa Laval pasteurizer simulating large commercial models was fitted for use and CIP (clean-in-place) cleaned according to common dairy industry practices. The standard soiling process was to maintain for 30 min a continuous pasteurization process at 72 C. The cleaning compounds were "phosphated caustic" (90% NaOH and 10% Na_3PO_4), two similar commercial highly alkaline detergent

formulations containing polyphosphates (exact composition unknown), and a commercial acid cleaner (primarily H_3PO_4). Rinsings were well beyond a visibly clean solution at the outlet of the system. The cleaning cycles were 20 min at 72 C.

RESULTS

Establishing a Standard Curve for Quantitation of Protein

Preliminary experiments indicated that the test for protein was influenced by constituents in tap water and some breakdown of gasket materials in the CIP system. Thus, the CIP system was cleaned by circulation and by hand to establish a starting point. A test-cleaning solution of 1% phosphated caustic then was circulated for 20 min at 72 C. This solution served as a carrier to which various quantities of milk protein in the form of nonfat dry milk were added. These samples were analyzed as described in Methods. The average results with 33 samples are in Figure 1 where it is apparent that the test is sensitive in the nanogram range. The intercept indicates the magnitude of the reactants from constituents in the local tap water and breakdown products of gasket materials.

To determine if most of the protein was

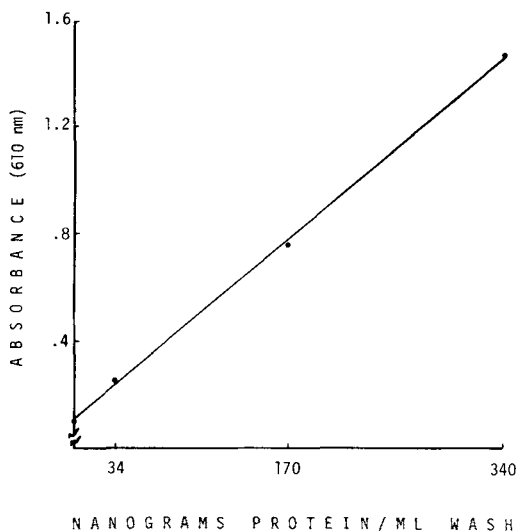


FIG. 1. Standard curve to relate protein in a test-wash solution to absorbance of the destain solution.

TABLE 1. Comparison of cleaner regimen.

Cleaner regimen	First test wash		Second test wash	
	Absorbance	Nanograms of protein/ml wash	Absorbance	Nanograms of protein/ml wash
A	2.00 (1.05) ^a	>500	1.31	300
B	2.00 (.57) ^a	>500	.89	190
C	1.09	241	.69	139
D	1.24	284	.57	112

^a Absorbance readings after a 1:10 dilution.

being swept down by the precipitation and flocculation process, test solutions were re-examined by adding calcium salts equivalent to the hard water constituents and then repeating the entire test system. The results indicated all the detectable protein was removed by the first precipitation and flocculation process.

Evaluation of Cleaner Effectiveness

A standard soiling of the pasteurizer was used to evaluate each of the four following cleaning regimen: A) phosphated caustic, B) phosphated caustic followed by an acid cycle, C) commercial caustic compound containing polyphosphates from formulator "C", and D) a commercial caustic compound with polyphosphates from formulator "D".

After each soiling and regimen of washing and rinsing, a test wash then was made, and the concentration of protein in the test-wash solution was determined. Further, a second test wash was made. The entire process of soiling, cleaning, and two test washings was repeated for a duplicate run. The results are in Table 1.

Each of the four cleaning regimen produced "clean" equipment as judged by disassembly of the plate pasteurizer and visual inspection. Results of the concentration and protein detection test, however, indicated differences in effectiveness of the four regimen. The efficacy of the test procedure in quantitating differences in performance of the cleaning regimen, especially C and D, was an important finding of this research.

DISCUSSION

The effectiveness of the test system is dependent on interaction of hard water with orthophosphates under alkaline conditions.

The precipitate of salts of phosphates along with the protein is flocculated for rapid sedimentation. This procedure provides approximately a 250-fold increase in concentration of the protein. The test-wash solution contained Na_3PO_4 and hard water constituents. If tap water is soft, some calcium salts should be added to the test-wash solution.

Acceptable sanitation in the dairy industry is based on routine cleaning beyond visual detection. A "safety" factor of additional cleaning commonly is applied. This procedure is a common waste of resources. In a process with an indeterminate safety factor, there is no way to measure the proper endpoint of cleaning to avoid microbial harborages or to assess the degree of waste from overcleaning. Thus, it is apparent that a highly sensitive test for cleaning effectiveness would be helpful for establishing parameters for industry processes and criteria for regulatory control.

A system depending on the criterion of visibly clean equipment as an endpoint assumes a positive correlation between removal of visible soil and removal of nutrients for microorganisms. This assumption is true only in a limited sense because with visibly clean equipment there is enough residue under certain conditions for microbial growth (5). Furthermore, certain cleaning regimen may be rapid and effective in removing visible soil but ineffective in removing microcontaminants.

The test previously described should be useful in studying and monitoring many fundamental and commercial practices of CIP regimen. In this research, comparison of some commonly used detergent systems showed definite differences in their effectiveness. Thus, with this methodology various detergent systems can be evaluated. By making the

detergent system a control, other cleaning variables such as equipment design, flow rate, temperature, and time could be studied. The test is sufficiently sensitive to quantitate the extent of cleaning in systems now judged visibly to be completely clean. The test is relatively simple, particularly if terminated at visual evaluation of the stained discs. This accuracy should be adequate for most purposes.

The observation that there was breakdown of gasket materials during cleaning and test washing did not pose a special problem in this work. However, the observation of sensitivity of the test system to breakdown of gaskets presented an idea for a method for evaluating gasket materials under use conditions. Work along these lines is now in progress.

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REFERENCES

- 1 Abele, C. A. 1965. Needed a reliable field determinant of cleanliness. *J. Milk Food Technol.* 28:257.
- 2 Arnold, R. G., and R. B. Maxcy. 1970. Evaluating circulation cleaning by analysis of soil depletion from surfaces. *J. Dairy Sci.* 53:1540.
- 3 Bramhall, S., N. Noack, M. Wu, and J. R. Loewenburg. 1969. A simple colorimetric method for determination of protein. *Anal. Biochem.* 31:146.
- 4 Kulkarni, S. M., R. B. Maxcy, and R. G. Arnold. 1975. Evaluation of soil deposition and removal processes: An interpretive review. *J. Dairy Sci.* 58:1922.
- 5 Maxcy, R. B. 1971. Factors in the ecosystem of food processing equipment contributing to outgrowth of microorganisms on stainless steel surfaces. *J. Milk Food Technol.* 34:569.
- 6 Maxcy, R. B., and R. G. Arnold. 1978. A test to determine microquantities of milkfat in cleaning solutions by surface film displacement. *J. Food Prot.* 41:14.
- 7 Maxcy, R. B., and K. M. Shahani. 1961. Cleaning and sanitization of welded line systems for handling milk. *J. Milk Food Technol.* 24:122.
- 8 Olson, H. C., G. O. Brown, and J. B. Mickle. 1960. A study of welded lines for processing milk. *J. Milk Food Technol.* 23:86.