Paper # NABEC/CSBE 12-063

Electrolyzed Oxidizing Water for Cleaning-In-Place of Milking Systems on Dairy Farms – Performance Evaluation and Assessment

Xinmiao Wang, Satyanarayan R.S. Dev, Ali Demirci,

Robert E. Graves, and Virendra M. Puri

Department of Agricultural & Biological Engineering

Pennsylvania State University

University Park, PA 16802

Abstract. The cleanliness of on-farm milking systems directly affects the raw milk quality. A four-step procedure is the generally accepted method for cleaning-in-place (CIP) milking systems: (1) lukewarm water rinse, (2) alkaline cleaning, (3) acid rinse, and (4) sanitizer circulation. Electrolyzed oxidizing (EO) water is a novel technology in which acidic EO water and alkaline EO water are generated separately by electrolyzing diluted salt solution within an electrified chamber with a membrane to partition the alkaline and acidic EO waters. As these solutions fit perfectly with the basic requirements for CIP of milking systems, it was proposed that EO water can be used as a cleaning and sanitizing agent for CIP of milking systems. Previous studies in our lab showed that the utilization of EO water for CIP provided equal or better results than conventional cleaning on a pilot-scale milking system. The current project is undertaken to evaluate, assess, and validate this technology at a commercial dairy farm compared with conventional method of CIP. Results show that EO water CIP performance is as good as or better than conventional CIP for most of the sampling locations and system components. This indicates that EO water can be adapted as an alternative CIP method for dairy farms.

Keywords. Electrolyzed Oxidizing (EO) water, Cleaning-In-Place (CIP), Milking Systems

A Community of

American Society of Agricultural and Biological Engineers

For presentation at

NABEC-CSBE/SCGAB 2012 Joint Meeting and Technical Conference
Northeast Agricultural & Biological Engineering Conference
Canadian Society for Bioengineering
Lakehead University, Orillia, Ontario
July 15-18, 2012

Introduction

Milking systems Cleaning-in-place (CIP) process, usually consisting of four steps, starts with a lukewarm water rinse, followed by an alkaline wash, then an acid rinse, and ends with one EPA-registered sanitizing cycle about one hour before the next milking. The lukewarm water rinse (45℃) helps to remove the milk residual from the milking system. During the alkaline wash cycle, by using a chlorinated alkaline detergent solution at the temperature of about 75℃, fat and protein deposits are removed from the milking system. The lukewarm or cold acid solution with a pH around 3 is used to neutralize the alkaline solution, remove the mineral deposits and leave the system in an acidified state to inhibit bacteria from growing (Table 1). The final sanitizing cycle is done about an hour prior to the next milking to destroy any remaining microorganisms.

Table 1: CIP Recommendations from Dairy Practice Council (DPC 4, 2010).

Cleaning Cycle	Conventional CIP
Lukewarm water rinse	2 minutes; 43.3°-48.9°C
Alkaline Wash	8-10 minutes; start:71.1°-76.7°C; finish:48.9°C; pH >12.0; 120 ppm chlorine; 1100 ppm alkalinity; >20 slugs
Acid rinse	3-5 minutes; pH~3.0
Sanitize	EPA registered dairy sanitizer solution

Electrolyzed oxidizing (EO) water is a novel technology in which acidic and alkaline EO waters are generated simultaneously by electrolyzing weak sodium chloride solution (0.1%) within an electrified chamber with a selective membrane between the anode and the cathode (Figure 1). Under certain driving voltage and amperage, acidic EO water has a pH as low as 2.6 and an Oxidizing Reducing Potential (ORP) as high as 1150 mV with the free chlorine content of 80 ppm, whereas the pH of the alkaline EO water can reach as high as 11.5 with an ORP of -850 mV (Fabrizio et al., 2002).

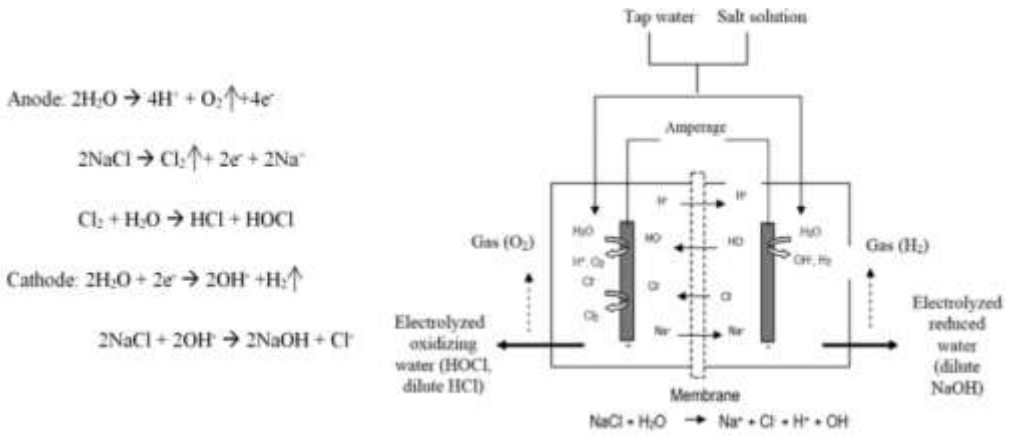

Figure 1: Schematic of the mechanism of EO water generation (Huang et al., 2008).

Previous studies showed that EO water has the potential to be utilized for effective cleaning and sanitizing of the fresh product and food preparation surfaces (Sharma et al., 2003; Ozer et al., 2006). But few studies focused on using EO water to clean the milking system, given that the properties of EO water solutions fit the basic requirements of milking system CIP. Using EO water to clean milking systems has advantages over using conventional CIP chemicals. The concentrated form chemicals used in conventional CIP cleaning possess potential hazards if

handled inappropriately, while EO water is not harmful to the operator for short time exposure. Considering this advantage, it was proposed that EO water can be utilized as alternative CIP solutions for milking system.

Previous studies in our lab showed the cleaning effectiveness by using EO water to clean a lab-scale pilot milking system (Walker et al., 2005a and b). An optimal temperature for effective CIP of milking systems was established with a logarithmic mean temperature of 58.8℃ and 37.9℃ for the alkaline and acidic EO water, and this model was validated for both short and long term evaluations using the pilot milking system (Dev et al., 2011). However, the lab-scale pilot system cannot represent a real commercial dairy farm CIP; therefore the current study was conducted on a commercial dairy farm to evaluate the EO water cleaning performance for milking system CIP.

Materials and Methods

Preparation of EO water

EO water is generated with a pilot-scale EO water generator (Figure 2, Model ROX60SA, Hoshizaki Electric Co. Ltd, Sakae, Toyoake, Aichi, Japan). Sodium chloride solution is generated automatically within the cell as long as the table salt is filled in the front chamber. By adjusting the voltage and amperage of the generator, the acidic EO water had (1) a pH of around 2.6, (2) an ORP around 1150 mV, (3) a free chlorine content of 80 ppm, whereas the pH of the alkaline EO water was 11.5 with an ORP of -850 mV. The pH and ORP of both solutions were examined by using a pH/ORP meter (Model 445, Corning, Inc., Big Flats, NY), and chlorine content of the acidic EO water was tested by using the titration with an *N, N*-diethyl-*p*-phenylenediamine-ferrous ethylene diammonium sulfate (DPD-FEAS) test kit (Hach, Inc., Loveland, CO). Alkaline EO water solution was heated using a tank heater (Model RUE PRO-80-2, Ruud Manufacturing Company, Atlanta, GA) and acidic EO water solution was heated using a tankless heater (Model EX1608TC, Eemax Inc, Oxford, CT).

Figure 2: Pilot-scale EO water generator.

Farm Trial

Farm trial was carried out on a commercial dairy farm, with 81 cows (Figure 3), 24 km from Penn State University. The 4-month effective evaluating session was divided into three periods: using the conventional milking system CIP as the baseline control for one month; using EO water milking system CIP to conduct the experiment for two months; and one more month of monitoring of conventional CIP process, making sure that no potential hazard was brought to the milking system during EO water CIP process. This trial was conducted with the permission of Pennsylvania Department of Agriculture.

Figure 3: Farm overview with milking and cleaning pipelines.

This 81-cow commercial dairy farm with pipeline length around 140 m is considered as a common dairy farm. The milk quality ranks above normal level, meaning that this farm is eligible to be a representative of typical commercial dairy farm in Central Pennsylvania. Detailed cleaning conditions are listed in Table 2.

Table 2: Cleaning condition comparisons between EO water CIP and conventional CIP on a commercial dairy farm.

Cleaning Cycle	EO Water CIP	Conventional CIP
Lukewarm water rinse	5 minutes start: 37 °C	5 minutes start: 36 °C
Alkaline Wash	10 minutes start: 72 °C finish: 42 °C pH 11.5 80 ppm chlorine	10 minutes start: 65 °C finish: 42 °C pH 11.5 125 ppm chlorine
Lukewarm water rinse	5 minutes 37 °C	-
Acid rinse	8 minutes start: 40 °C finish: 20 °C pH 2.6	7.5 minutes start: 18 °C finish: 15 °C pH 2.5

Lukewarm water rinse was used before and after alkaline solution washing cycle during EO water CIP, while only one lukewarm water rinse cycle before alkaline solution washing was used during conventional CIP process. Conventional CIP chemical solutions used in this farm are:

sodium hydroxide/sodium hypochlorite mixture of alkaline solution (Liquid Pfite, GEA WestfaliaSurge Inc, Naperville, IL) and phosphoric/sulfuric blend of acid solution (Dairy Star CIP Acid, GEA WestfaliaSurge Inc, Naperville, IL). Conventional CIP process utilized alkaline cleaning solution with a starting temperature of around 65℃ and unheated (room temperature) acidic rinsing solution. Alkaline EO water solution was heated up to around 70℃ before usage, and acidic EO water solution was heated up to around 40℃, both of which are recommended by the Dairy Practice Council (DPC 4, 2010).

Sampling Sites

Nine sampling locations at tri-clamp connections along the stainless steel milking and also cleaning system pipelines were chosen. Detailed information about these nine sampling sites is shown on Figure 4.

These nine sampling locations along the milking and also cleaning system pipelines can be divided into three categories: Locations A and B both have 45° elbows with vacuum and slugs during cleaning process; Locations C, D, and E all have 90° elbows with vacuum and slugs during cleaning process; the rest of them, namely Locations F, G, H and I have 90° elbows did not have vacuum nor slugs during cleaning process.

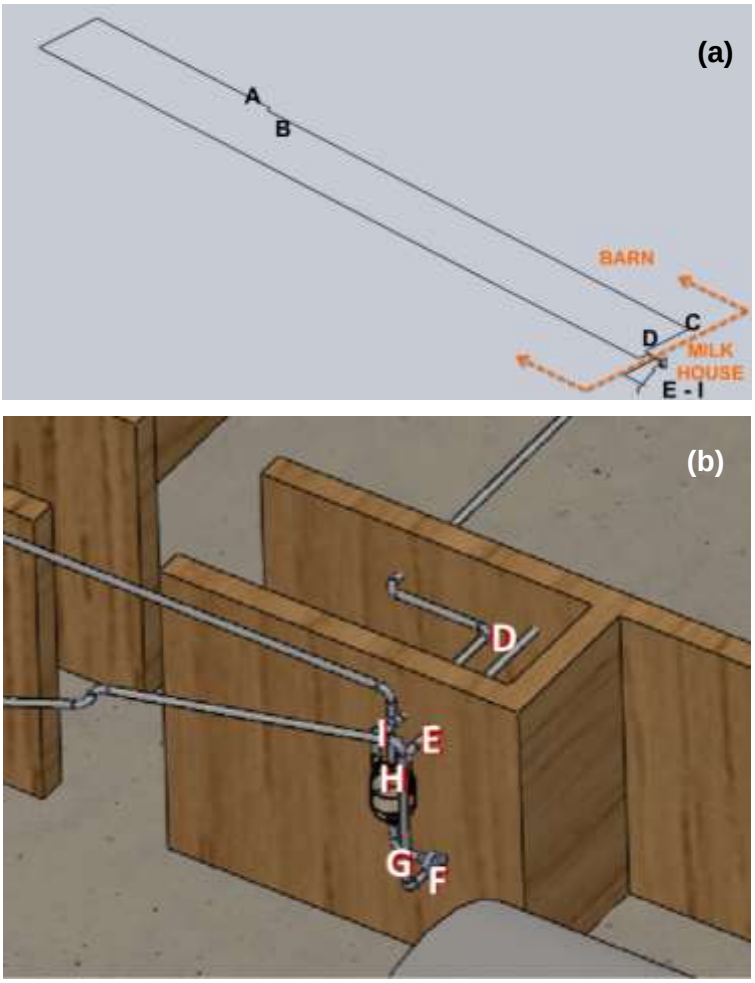

Figure 4: General systematic overview of farm milking and cleaning system (a); detailed sampling locations along the pipelines in milk house (b).

In addition to these sampling locations along the cleaning system pipelines, other milking system components, namely liners, milk hoses and milk inlets were also analyzed on a regular basis.

Sampling Protocol

The nine sampling locations plus other components were analyzed for ATP Bioluminescence and bacteria presence. Samples were collect from the nine sampling locations along the system pipelines with tri-clamps, by disassembling the connections and swabbing the inner surfaces of elbows and straight pipelines. Gaskets in the middle of the tri-clamp were sampled as well.

Based on the cleaning solution flow direction, the right half of the connections of straight pipeline, elbow and gasket was swabbed using ATP swabs and the left half using sterile calcium alginate tipped applicators (Figure 5 and 6). Other components of liners, milk hoses and milk inlets were sampled (Figure 5 and 6) likewise; the only difference was that these components were swabbed over the entire circle instead of the half circle due to the limited swabbing areas. On the sampling day, three randomly assigned sampling locations out of nine were sampled following the sequence of A, F, H; B, C, E and D, G, I. At the same time, three liners, three milk hoses and three milk inlets were used for ATP sampling and another three of each category were used for bacteria enrichment in random order.

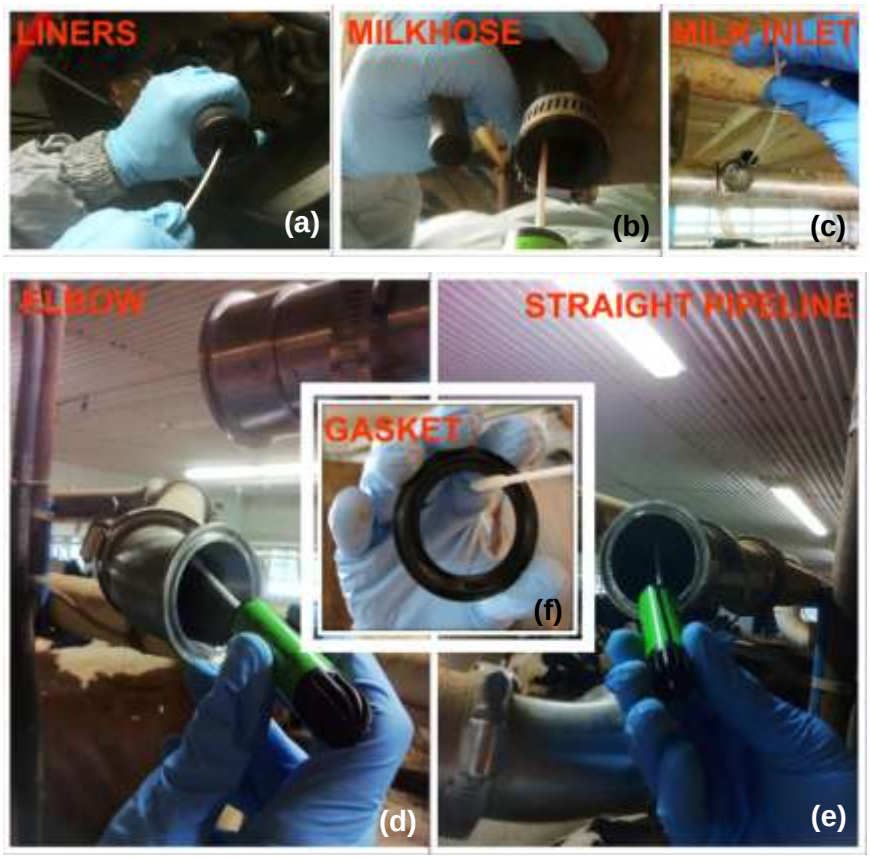

Figure 5: Sampling protocols for different sampling sites. liner sampling (a); milk hose sampling (b); milk inlet sampling in the barn (c); dissembled elbow sampling at one sampling location along system pipeline (d); dissembled straight pipeline sampling at one sampling location along system pipeline (e); gasket sampling at a sampling location along system pipeline (f).

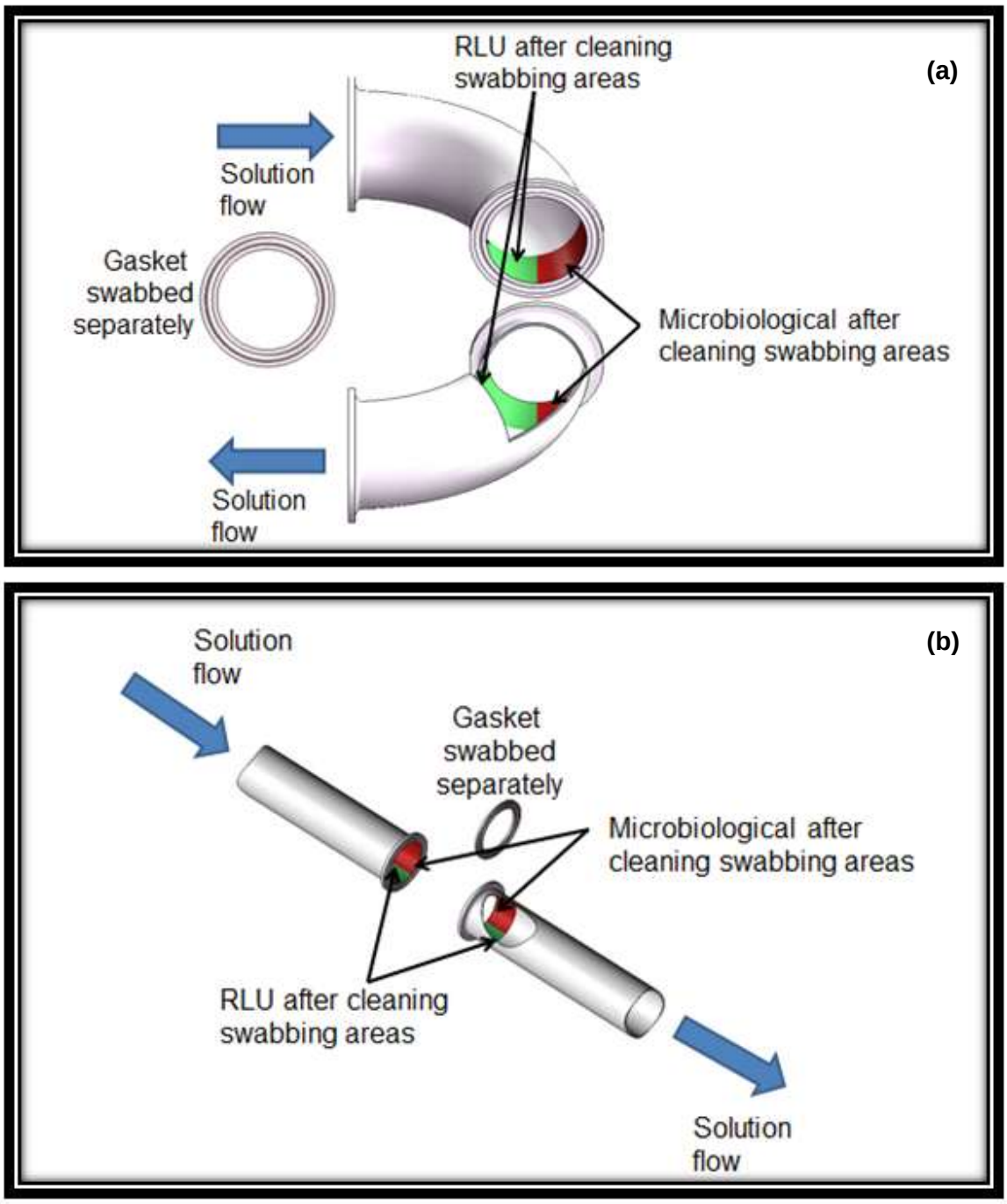

Figure 6: Sampling schematic protocol. Elbow sampling (a); Straight pipeline sampling (b).

Analysis

Inner surfaces of all sampling sites were sampled using PocketSwab Plus swabs (Charm Science, Inc., Lawrence, MA) and sterile calcium alginate tipped applicators (Puritan Medical Production Company LLC, Guilford, ME) (Figure 7). The measurement of ATP swabs' firefly enzyme luciferase resulted in a quantitative Relative Light Unit (RLU), which served as an indicator of the surface cleanliness. This was completed by using a novaLUM palm-sized luminometer (Charm Science, Inc.). Sterile calcium alginate tipped applicators swabbed samples were enriched in a Tryptic Soy Broth (TSB) medium and incubated for 48 hours. As the common microbiological observation, the transparent enrichment observed visually were defined as "Negative" while the opaque (turbit) as "Positive". Negative enrichment percentage is calculated by using negative sample number divided by all sample number (negative sample plus positive sample numbers) for each sampling site.

Figure 7: Sampling indicators used: ATP Bioluminescent Relative Light Unit (RLU) readings and bacteria presence enrichment data. ATP swab (a); ATP laminator (b); positive (turbit) bacteria enrichment (c); negative bacteria enrichment (d).

Results and Discussions

ATP RLU Readings

ATP test is a sensitive test; the RLU readings vary from zero to millions depending on the status of the surfaces. RLU reading of zero represents the surface to be clean and the higher the RLU reading the "dirtier" the surface is. A practical cut-off of RLU reading is 1,000 for stainless steel (namely elbow, straight pipeline, and milk inlet in this study) and 4,500 for porous rubber material (liner and milk hose in this study). A logarithm transformation was used to adjust the large-ranged results to be normally distributed for better statistical analysis (Figure 8). By using"RLU+1" the minus infinity is avoided and 1 RLU reading has negligible effect on the transformations and comparisons of the results.

In general, the average RLU readings of most sampling sites including sampling locations and system components were lower than the cut-off values. That indicated the milking system was working well without any potential hazard involved during the evaluation and comparison process.

For gaskets among all the sampling locations, most average RLU readings were below the cut-off value using both methods. EO water RLU average readings were lower than conventional RLU average readings, but not statistically different.

For elbows and straight pipelines, most of the average RLU readings were below the cut-off value using both methods, too (except for location H, which will be discussed below). EO water RLU average readings were lower than conventional RLU average readings (for example, conventional sampling location C straight pipeline log (RLU+1) was 1.603 and EO water sampling location C straight pipeline log (RLU+1) was 1.535).

There were some sampling locations where EO water RLU average readings were higher than conventional RLU average readings, but none of them were statistically different. In other words, EO water CIP performance is statistically “not worse” than conventional CIP.

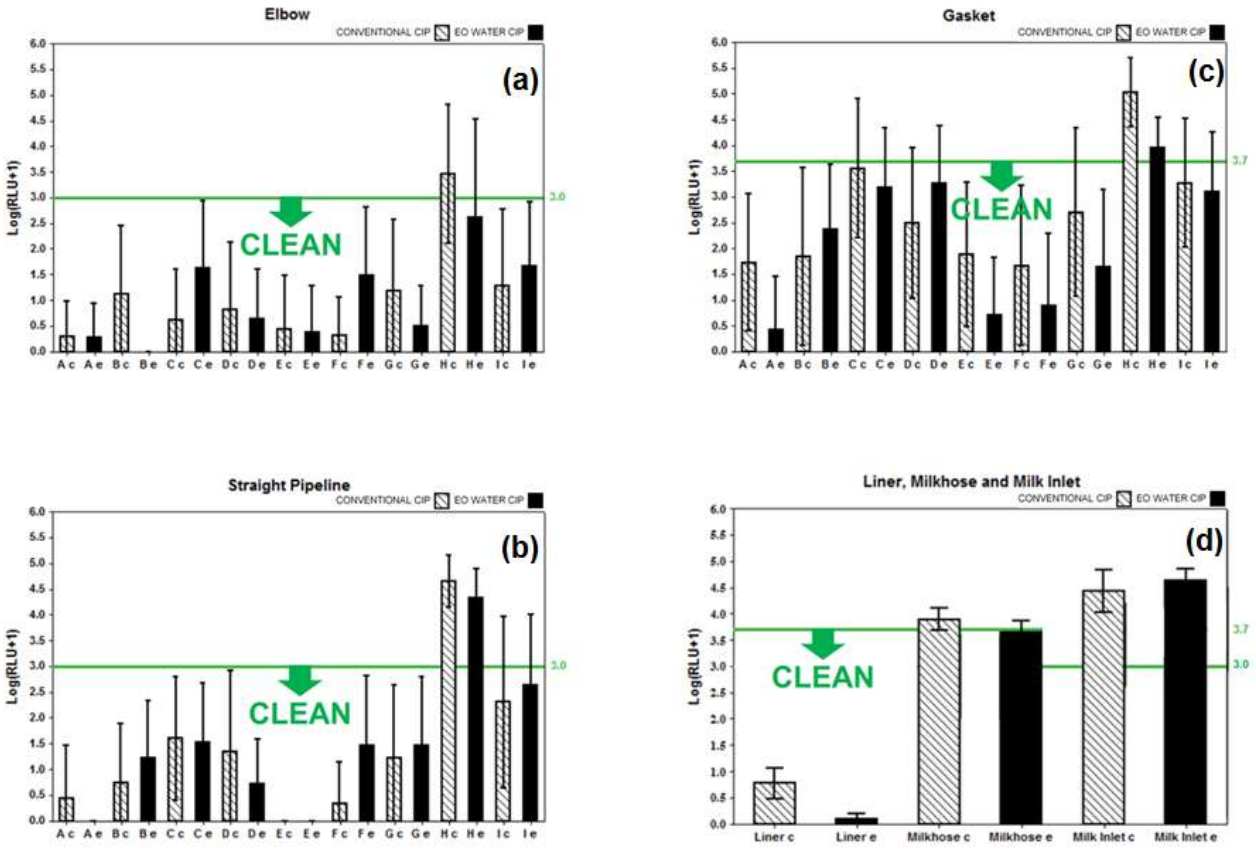

Figure 8: Average RLU reading comparison between EO water CIP and conventional CIP with respect to elbow (a), straight pipeline (b) and gasket (c) for sampling locations and liner, milk hose and milk inlet for other components (d).

As is shown in Figure 8, the average RLU reading for liner when using EO water CIP was significantly lower ($P<<0.05$) than conventional CIP (conventional CIP with the log(RLU+1) to be 0.783 and EO water 0.091). The average RLU reading for milk hose when using EO water CIP was lower than conventional CIP, but not statistically different ($P>0.05$). The average RLU reading for milk inlet when using EO water CIP was higher than conventional CIP, but readings from both methods exceeded the cut-off value of 1,000. This is caused by the fact that milk inlet along this farm system pipeline is set at an angle towards the ceiling (Figure 7(c)) which makes it very difficult for the milk inlet inner surface to ever be cleaned thoroughly as the result of the gravity of the cleaning solution, even with the presence of vacuum and slugs.

For the nine sampling locations (A-H), the gasket RLU readings were higher in general than the elbow and straight pipeline RLU readings. This was caused by the material of the gaskets.

Gaskets were mostly made of porous rubber which might be pitted by the powerful cleaning solution with usage.

The explanation for location H high RLU readings comes from the configuration of this location. Location H had neither vacuum nor slugs during cleaning process for both cleaning methods. When the cleaning process finished, vacuum shutting down, cleaning solution would remain in the vertical pipeline where H located without being drained through either the milk transfer pump or the vat. This led location H to be the horizontal level of the leftover cleaning solution. Cleaning waste floating on top of the remaining solutions led H site “dirty” for both methods.

Microbiological Enrichment

Microbiological analysis was conducted by using simple enrichment (Figure 7). Negative enrichment percentage is calculated by using negative sample number divided by all sample numbers (negative sample plus positive sample numbers) for each sampling site. Due to the schematic plan of the sampling procedure, not all of these nine sampling locations share the same number of sampling times. Detailed comparisons can be found in Figure 9.

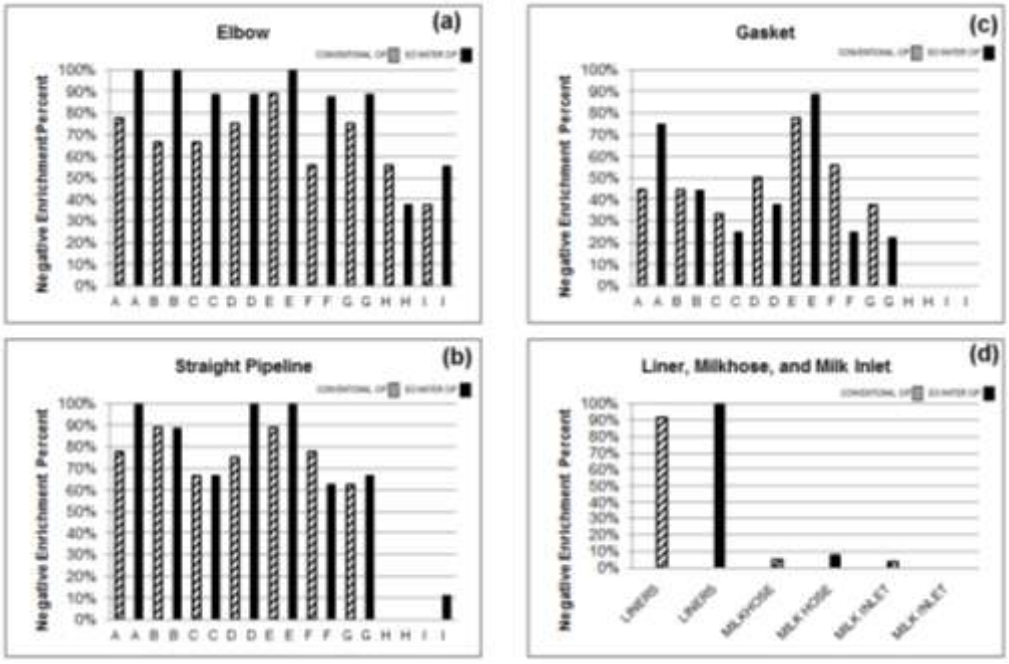

Figure 9: Negative bacteria enrichment comparison between EO water CIP and conventional CIP with respect to elbow (a), straight pipeline (b) and gasket (c) for sampling locations and liner, milk hose and milk inlet for other components (d).

Based on the definition of “negative enrichment percentage”, it is easily seen that higher negative enrichment percentage represents less microorganism presence. Clearly for most of the nine sampling locations along the milking system pipeline, the negative enrichment percentages of using EO water CIP were higher than using conventional CIP (for example, conventional sampling location B negative percentage was 67% and EO water sampling location B negative percentage was 100%). Some locations like A and E gave the negative enrichment of using EO water CIP 100% for both elbows and straight pipelines, 10% or higher than conventional CIP. In the meantime, corresponding to the discussion above about location H, the bacteria enrichment results showed that at this location the negative enrichment percentages are both zero for straight pipeline and gasket using both methods. This result

indicated that at this particular location, no matter what type of solution was being used, no effective method took place on preventing the bacteria from growing.

For other components of the liners, milk hoses and milk inlets, the negative percentages enrichment of EO water CIP were higher than conventional CIP (for example, conventional milk hose negative percentage was 5% and EO water milk hose negative percentage was 8%). These results also correspond to the previous ATP RLU reading results. Microorganism grew more actively within the surfaces of milk hoses and milk inlets - one came from the porous material and the other one came from the direction of milk inlet setting. Fewer microorganisms built up on the surfaces of liners because liners were immersed with cleaning solution for much longer time duration. In addition, during the very important lukewarm water rinse cycle, water coming back from the pipelines was drained directly into the small vat instead of recirculating and contaminating the liners again. This is another reason for the liners ATP RLU readings to be lower.

Conclusion

This study conducted on the commercial dairy farm aims to evaluate and assess the cleaning performance between using acidic and alkaline EO waters and using conventional cleaning chemicals for milking system CIP. The four month trial reached the conclusion that EO water achieved same or better cleaning effectiveness compared with conventional CIP solutions on this representative commercial dairy farm for most sampling locations and milking system components.

Previous studies of using EO water to clean produce surfaces laid a solid scientific foundation, and this current study further expanded the application of EO water - EO water is a promising technology for milking system CIP.

To further promote and develop this technology of using EO water for milking system CIP, more work should be done to study the stability of this technology in the long run. In addition, computational simulations are necessary in terms of reducing the labor and time and bringing more profits.

Acknowledgement

Funding for this project was provided by USDA Special Research Grant and the Pennsylvania Agricultural Experimental Station. Special thanks go to the Peachey family for letting us use their milking system. We also thank Hoshizaki Electric Co. Ltd., Charm Science Inc., and Fisher and Thompson Inc. for the technical help, and we would like to deliver our thanks to Dr. Roderick Thomas, Mr. Randall Bock, Robin Pritz and Meghan Fisher for their valuable technical advice and help.

Reference

Dev, S. R. S., A. Demirci, and R. Graves. 2011. Mathematical modeling of CIP of milking systems using electrolyzed oxidizing water. *NABEC*:11-016

DPC. 2010. Guidelines for installation, cleaning, and sanitizing of large and multiple receiver parlor milking systems. *Dairy Practices Council Publication* Number:4,Feb 2010

Fabrizio, K. A., R. R. Sharma, A. Demirci, C. N. Cutter, 2002. Comparison of Electrolyzed Oxidizing Water with Various Antimicrobial Interventions to Reduce *Salmonella* Species on Poultry. *Poultry Science* 81: 1598-1605

Huang, Y. R., Y. C. Hung, S. Y. Hsu, Y. W. Huang and D. F. Hwang. 2008. Application of electrolyzed water in the food industry. *Food Control* 19:329-345

Ozer, N. P., A. Demirci. 2006. Electrolyzed oxidizing water for decontamination of raw salmon inoculated with *Escherichia coli* O157:H7 and *Listeria monocytogenes* Scott A and response surface modeling. *J. of Food Eng* 72:234-241

Sharma, R. R., A. Demirci. 2003. Treatment of *Escherichia coli* O 157: H7 inoculated alfalfa seeds and sprouts with electrolyzed oxidizing water. *Int J. Food Microbiology* 86:231-237

Walker, S. P., A. Demirci, R. E. Graves, S. B. Spencer, and R.F. Roberts. 2005a. Cleaning milking systems using electrolyzed oxidizing water. *Transactions of ASAE* 48:1827-1833.

Walker, S. P., A. Demirci, R. E. Graves, S. B. Spencer, and R.F. Roberts. 2005b. Response surface modeling for cleaning and disinfecting materials used in milking systems with electrolyzed oxidizing water. *Society of Dairy Technology* 58(2):65-73