Cleaning Milking Systems Using Electrolyzed Oxidizing Water

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Abstract. *Electrolyzed oxidizing (EO) water is a novel cleaning and disinfecting agent, produced by separating a weak sodium chloride solution into alkaline and acidic components. A pilot-scale pipeline milking system was soiled using raw milk inoculated with common microorganisms. The milking system was then washed with alkaline EO water followed by acidic EO water. After cleaning, the effectiveness of the EO water treatment was evaluated by ATP bioluminescence and microbiological analysis. A 10 min wash with 60°C alkaline EO water followed by a 10 min wash with 60°C acid EO water successfully removed all detectable bacteria and ATP from the non-porous milk contact surfaces. Shorter treatment times (5 and 7.5 min) with EO water were also evaluated, along with a control treatment using conventional dairy cleaning chemicals. There were no significant differences between the 10 min and 7.5 min EO water treatments and the conventional treatment. In a longer-term soiling-washing simulation, only the 7.5 min EO water treatment was evaluated after ten soiling and cleaning cycles, and it was*

compared with a conventional cleaning treatment. The 7.5 min EO water treatment did not acceptably clean the milking system, but longer treatment times were not attempted. Overall, results indicate that EO water has the potential to be used as a cleaning and sanitizing agent for CIP cleaning of on-farm milking systems.

Keywords. ATP bioluminescence, Electrolyzed oxidizing water, EO water, Milking systems.

There are tens of thousands of dairy farms across the country (NASS, 2000). Most of these facilities use some form of mechanized milking system that collects the milk through a series of pipes to a central refrigerated holding tank. According to regulations, the milking equipment must be cleaned and sanitized regularly (PHS/FDA, 1999).

In the U.S., the law requires that the bacterial population of raw milk intended for pasteurization be less than 100,000 CFU/mL standard plate count (PHS/FDA, 1999). Milk processors often pay premiums for higher-quality milk, and the Dairy Practices Council (DPC) recommends a standard plate count of less than 5,000 CFU/mL as a guideline when producing high-quality milk (NDPC, 1993). Good cleaning practices help ensure production of high-quality milk, leading to higher profitability for the producer.

Most pipeline milking systems share similar design features. The interface to the individual animal is the milking unit. The milking unit consists of a cluster of four teatcups and a claw (*ASAE Standards* , 2003). Each teatcup is made up of a rigid shell with a rubber liner inside. The rubber liners are connected to the claw, which is simply a manifold that connects the four teatcups to the pipeline. The pipeline, or milkline, carries the milk from the milking unit to the receiver. The pipeline is typically stainless steel and can range from about 1.5 to 4 inches in diameter depending on the size and capacity of the milking system. In a pipeline milking system, the pipeline extends around the barn and the cows are milked at their stalls. In such a system, the milking units are moved from animal to animal and connected to the pipeline via an opening in the pipeline called a milk inlet or nipple. The receiver, typically glass, is a vessel that receives milk from one or more pipelines and connects to both the vacuum supply and the milk pump. The milk pump, or releaser milk pump, removes milk from the receiver under vacuum and discharges it at atmospheric pressure.

Pipeline milking systems are usually cleaned using a two-phase flow of air and water referred to as "slug" flow (Reinemann, 1995). This requires the use of an air injector, that is, a valve that introduces air into the system near a wash manifold, where the milking units are connected for cleaning. Cleaning solutions are drawn into the pipeline through the milking units, and then the air injector opens and the atmospheric pressure pushes the "slug" of cleaning solution through the pipeline and into the receiver with substantial velocity. The cleaning solutions are pumped from the receiver with the milk pump and either recirculated or discarded.

Milking systems are commonly cleaned with a four-step process: a warm water rinse, washing with a highly alkaline solution, a rinse with an acidic solution, and then sanitizing with an EPA-registered sanitizing agent immediately prior to the next use (NDPC, 1993). The warm water (about 40°C) rinse removes most of the residual milk from the system. During the alkaline wash cycle, a chlorinated alkaline detergent solution at high temperature (80°C) removes fat and protein deposits from the system. The acid rinse, usually a weak acid solution (pH 3.5 to 4.5) at room temperature, serves to neutralize the alkaline cleaner, dissolve mineral deposits, and leave the system in an acidified state to retard bacteria growth. Chemicals used in cleaning milking systems are delivered to and stored on the farm in concentrated form. Both the concentrated alkaline cleaner and the concentrated acid can cause serious burns of the skin and eyes on contact.

Electrolyzed oxidizing (EO) water is a novel cleaning and sanitizing agent that has been shown to be an effective antimicrobial agent for fresh produce and food preparation surfaces (Izumi, 1999; Morita et al., 2002; Venkitanarayanan et al., 1999; Park et al., 2002). EO water is produced by passing an electric current between two electrodes separated by a membrane and immersed in a 0.1% sodium chloride solution. The sodium and chlorine ions are attracted to opposite electrodes, yielding an alkaline solution and an acidic solution. The alkaline solution has a pH of 11.5 and an oxidation-reduction potential (ORP) of -850 mV, while the acidic water has a pH of 2.6, an ORP of 1150 mV, and contains 50 to 80 ppm of free chlorine (Kim et al., 2001). Using EO water for CIP cleaning of milking systems eliminates many of the dangers associated with storing and using costly cleaning chemicals. EO water is not harmful to the skin for short exposure times. Since EO water has both alkaline and acidic components, it should fit easily into the accepted four-step washing process for CIP cleaning of milking systems by replacing the alkaline wash and acid rinse solutions with EO water solutions.

EO water also has the potential to be more cost effective than traditional cleaning agents. Chemicals used for cleaning are expensive and represent an operating expense for the dairy producer. Once the initial capital investment is made to purchase a machine to make EO water, the only operating expenses are water, sodium chloride (ordinary table salt), and electricity to run the unit.

With obvious advantages in terms of safety and the potential for a profit advantage due to decreased operating costs, EO water seems to have a potential for use in cleaning milking systems. A previous study showed that EO water could be used effectively to clean small pieces of common milking system materials by soaking them in alkaline EO water followed by acidic EO water (Walker et al., 2005). In the same study, a response surface model was developed that showed that all treatment parameters (alkaline treatment time, acid treatment time, and treatment temperature) were significant parameters. This model was used to help select the treatment times and temperatures used in this study. The present study attempted to extend the results of the laboratory study by cleaning a pilot-scale pipeline milking system, and evaluate the efficacy of EO water treatment for short-term and long-term cleaning.

Materials and Methods

Construction of the Milking System

A pilot-scale milking system with all the major components of a typical pipeline milking system was constructed (fig. 1). The milking unit (DeLaval, Kansas City, Mo.) was connected via a PVC milk hose and washing manifold to eight 10 ft (3.05 m) segments of 1.5 in. (3.94 cm) diameter sanitary pipe. The sections of sanitary pipe were joined with rubber gaskets and tri-clamps. The pipeline was mounted on a 20 ft (6.1 m) long steel frame, wrapping around the frame in a spiral fashion with using 90° elbows to join the pipe lengths at the ends of the frame. The pipeline was sloped at a rate of 1 in. every 10 ft to facilitate draining. A glass receiver jar and milk pump (DeLaval, Kansas City, Mo.) was also mounted on the steel frame. The receiver jar was connected to a regulated vacuum source via a stainless steel sanitary trap.

Before the experiments, the system was adjusted to provide acceptable flow dynamics for CIP cleaning. A two-channel vacuum recorder was used, as described by Reinemann (1995), to monitor slug velocity for the initial adjustment of the system. The timing and admission rate of the air injector was adjusted to achieve slug flow with a slug velocity of approximately 30 ft/s (9.1 m/s) and a delay of approximately 6 s between each slug.

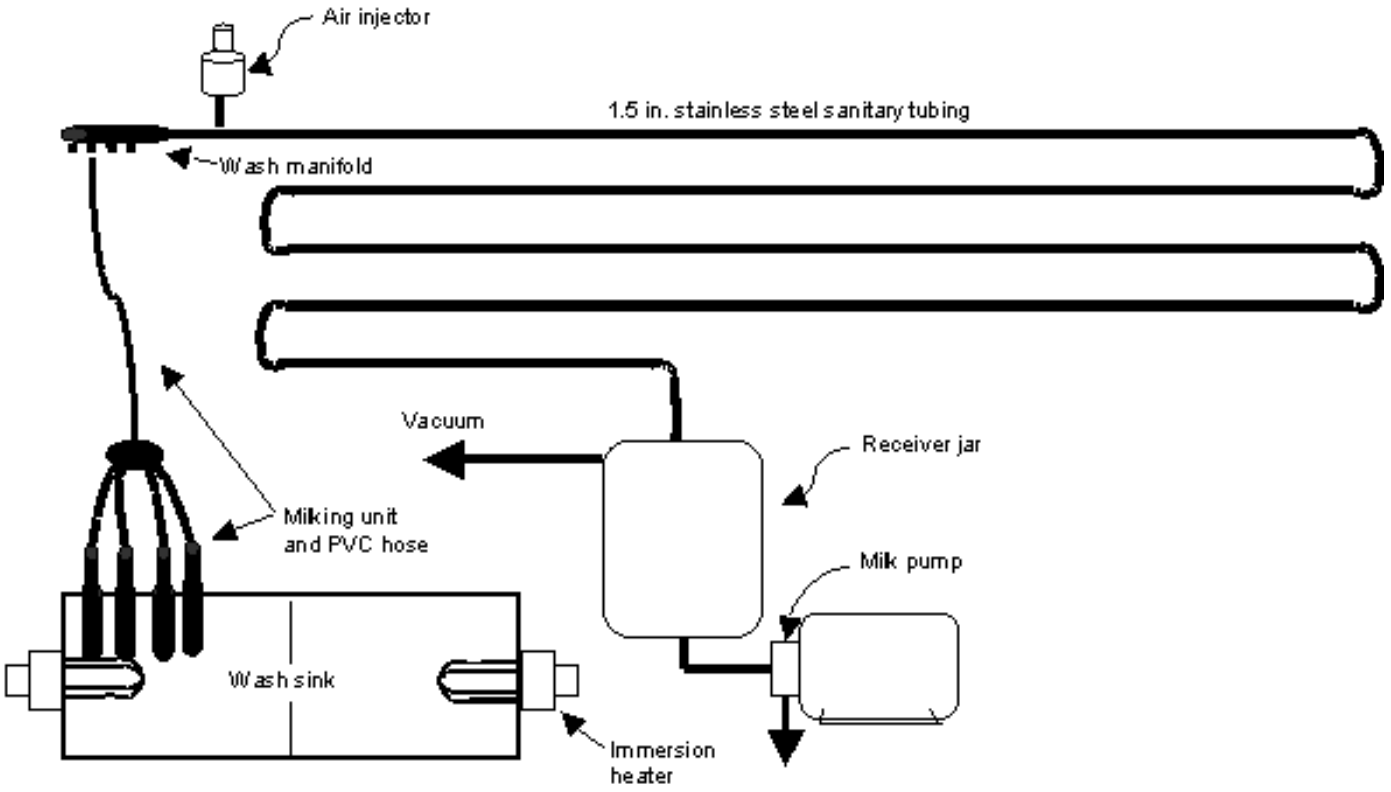

Figure 1. Schematic of the milking system.

Preparation of Milk

A cocktail of *Pseudomonas fluorescens* B2 (obtained from Dr. S. Doores, Department of Food Science, Pennsylvania State University), *Micrococcus luteus* (ATCC 10240), *Enterococcus faecalis* (ATCC 51299), and *Escherichia coli* (ATCC 25922) was used as the inoculum in this study to increase microbial population of raw milk. Each culture was grown in 500 mL of tryptic soy broth (TSB) (Difco, Sparks, Md.) for 18 h at its optimum temperature: 30°C for *P. fluorescens* and *M. luteus* , and 37°C for *E. faecalis* and *E. coli* . Microorganisms were present in each culture at a level of approximately 10^8 CFU/mL. Following growth, each culture broth was centrifuged at 4,400 × *g* for 40 min, the supernatant was decanted, and then the bacterial cells were re-suspended in about 250 mL of raw milk. Finally, this inoculum was then mixed with 10 gal (38 L) of raw milk that had been heated to 38°C to mimic the temperature of milk coming from a cow.

Design of the Experiment

To evaluate the efficacy of EO water treatment following one soiling cycle, three experiments were evaluated. The three experiments used EO water treatment times of 10, 7.5, and 5 min each for alkaline EO water and acidic EO water. A conventional treatment using conventional chemicals was also evaluated as a control. Each EO water treatment was replicated three times in succession. Before each set of replications, the system was prepared with a strong cleaning treatment to ensure clean initial conditions. For every experiment, the system was soiled with the prepared raw milk before each cleaning cycle and then evaluated to ascertain the initial condition. After each cleaning cycle, the milking system was evaluated to measure the effectiveness of cleaning and disinfection.

To evaluate the efficacy of EO water cleaning over multiple uses, the treatment time of 7.5 min was

selected based on the results obtained during the short-term evaluation. This long-term evaluation was designed to simulate using EO water to clean milking systems on a regular basis. For comparison, the conventional treatment was again used as a control. In these experiments, a regimen of ten soiling and cleaning cycles was performed with no disassembly or evaluation after individual cleanings, meaning that the system was only evaluated for post-treatment conditions following the tenth cleaning. All ten soiling-cleaning cycles were accomplished over three consecutive days, with four cycles on the first two days and two cycles on the third day. The EO water treatment protocol was replicated three times, and the conventional treatment was replicated twice.

Preparation of the System

Before each set of experiments, the system was cleaned to return the system to a presumably clean condition. The strong cleaning protocol consisted of a wash with a commercial sodium hydroxide-based cleaning solution (Dairy Cycle 3, Chemland, Inc., Kansas City, Mo.) supplemented with 100 g of lye (sodium hydroxide) to 10 gal of prepared cleaning solution. This cleaning solution was heated to a temperature of approximately 80°C before cycling it through the system for 10 min. This was followed by a 5 min phosphoric and sulfuric acid wash (Dairy M.S.R. 50, Chemland, Inc., Kansas City, Mo.) also starting at 80°C with 10 gal of acid wash solution. Before every soiling, the system was sanitized by rinsing with a sodium hypochlorite sanitizing solution (LCS, Classic Technologies, Kansas City, Mo.) diluted to 50 ppm concentration in 10 gal of water.

Soiling the System

Unlike an actual milking system, the milking unit of the test system was connected to the wash manifold during soiling. With the vacuum pump on, the milk was drawn through the entire length of the pipeline to the receiver, ensuring contact with all inside surfaces due to the highly turbulent flow. The inoculated raw milk was introduced into the system by inserting the milking unit into the container of milk. The 10 gal of milk was introduced into the system in three approximately equal portions, with a 10 min rest between each portion to allow the milk to adhere to the pipeline and other milk contact surfaces. The milk was not recirculated; it was pumped from the receiver and discarded.

Evaluation of the Initial Conditions

During the short-term tests, the initial soiling levels were evaluated. After a 10 min pause, the system was rinsed with 10 gal of clean warm (40°C) water to remove residual milk from the system. After rinsing, the system was allowed to drain for 10 min to avoid the variability of free water in the system. Then, the system was disassembled and swabbed in numerous locations using two methods: swabbing for microbiological analysis, and swabbing for detection of soil using an ATP bioluminescence method. For each method, eight locations on the milking system were swabbed: one on the inside of the plastic claw, one on the neck of the glass receiver, two on the inside of the rubber liners, two on the inside of the stainless steel elbows, and two on the inside of the stainless steel pipeline. To evaluate the surviving microorganisms, a sterile calcium alginate swab was used to swab an area of approximately 30 cm^2. To reduce variability, all swabbing was performed by the same individual.

Despite the high microbial load of the inoculated raw milk, numbers of bacteria recovered from the system immediately after the warm water rinse were lower than could be reliably counted using the standard plate count method. Therefore, to evaluate the presence of microorganisms on the milk contact surfaces, the swabs were placed in 10 mL of tryptic soy broth for enrichment purposes. The enrichment

cultures were incubated at 30°C for 48 h and visually evaluated. The presence of turbidity in the cultures following incubation was taken as evidence of viable microorganisms.

An ATP bioluminescence method was used to determine surface cleanliness. Approximately 30 cm^2 areas of the milking system were swabbed using a PocketSwab Plus (Charm Science, Inc., Lawrence, Mass.). The self-contained swabs contain the firefly enzyme luciferase, causing any collected ATP on the swab to emit light, which was detected and quantified into "relative light units" (RLU) by the LUMinator-T portable analyzer (Charm Science, Inc.). While the manufacturer does not claim any correlation to any physical measure of soil, a RLU value of zero represents an acceptably clean stainless steel surface (no detectable ATP). In a commercial setting, porous materials such as rubber may be deemed acceptably clean even if small amounts of ATP are detected (V. Saunders, Charm Science, Inc., Lawrence, Mass., personal communication, 2002).

Preparation of Electrolyzed Oxidizing Water

Electrolyzed oxidizing water was generated with an EO water generator (model ROX15SA, Hoshizaki Electric Co. Ltd., Sakae, Toyoake, Aichi, Japan). The generator used in this study was a self-contained unit including a water softener, salt reservoir, separate 200 L storage tanks for each alkaline and acidic EO water, and dispensing pumps. The electrolysis chamber was set to 19 A and 10 V, and the system automatically mixes salt and water to yield a 0.1% sodium chloride solution in the electrolysis chamber. The storage tanks were drained before each run and filled with fresh EO water.

Since the generator is supplied with cold water, the EO water was heated before use. For this purpose, each side of a double-bowl stainless steel wash sink was outfitted with a 3000 W stainless steel clad immersion heater and wrapped with fiberglass insulation to reduce heat loss. Each bowl was filled with 10 gal of alkaline or acid water and covered with an expanded polystyrene insulation board. Heating the EO water from its initial temperature of about 20°C to the treatment temperature of 60°C took about 40 min. After heating, paper test strips (Advantec MHS, Inc., Dublin, Cal.) were used to verify that the chlorine content of the acidic EO water was above 50 ppm and the pH of the alkaline EO water was above 11.0.

EO Water Treatment

For EO water treatments, the starting temperature for both the alkaline EO water and the acidic EO water washes was 60°C. For the short-term evaluation, the first treatment was a 10 min alkaline EO water wash followed by a 10 min acidic EO water wash, as suggested by a previous laboratory study (Walker et al., 2005). Shorter treatment times of 5 and 7.5 min were also evaluated. For the long-term evaluation, a 7.5 min EO water treatment was used. All EO water treatments were replicated three times.

After the warm water rinse, the alkaline EO water was introduced into the system by placing the milking unit into the sink. The milk pump on the bottom of the receiver jar pumped the alkaline EO water back to the sink. After the prescribed alkaline treatment time, the milk pump outlet was routed to the drain and the system emptied of the alkaline EO water. The milking unit and milk pump outlet were then placed into the sink bowl containing the acidic EO water. The system was drained at the end of the acid treatment time, and allowed to dry for 10 min before evaluation.

Conventional Treatment

A protocol utilizing conventional cleaning chemicals was used as a control for both the short-term and long-term evaluations. The model milking system was soiled and analyzed by methods identical to those described above for the EO water treatments. Instead of using EO water to clean the system, conventional chemicals were used as follows:

A chlorinated alkaline cleaner (Dairy Cycle 3, Chemland, Inc., Kansas City, Mo.), an acid rinse (Dairy M. S.R. 50, Chemland, Inc., Kansas City, Mo.), and sanitizer (LCS, Classic Technologies, Kansas City, Mo.) were used. All cleaning solutions were prepared and applied according to manufacturer's instructions. The chlorinated alkaline cleaner was diluted by mixing 5 ounces of the concentrate with 10 gal of 80°C water. The alkaline solution was circulated for 10 min and then drained from the system completely. The acid rinse was diluted by mixing 2 ounces of concentrate with 10 gal of cool water (20°C). The acidic solutions was circulated for 5 min and then drained from the system. Immediately following the acid rinse, the system was rinsed with a chlorinated sanitizing solution. The sanitizing solution was diluted to approximately 100 ppm available chlorine by mixing 2 ounces of the concentrated product with 10 gal of cold water (15°C). The sanitizing solution was not recirculated; it was pumped from the receiver and discarded. Paper test strips were used to verify that the pH of the cleaning solution was above 11.0 and the chlorine content of the sanitizer rinse was above 50 ppm.

For the short-term evaluation, the system was then disassembled and swabbed in similar locations as for the pre-treatment evaluation described earlier. For the long-term evaluation, the system was soiled and cleaned ten times before it was disassembled and swabbed. The conventional treatment for the long-term evaluation was repeated twice.

Statistical Analysis

The ATP bioluminescence data for all three EO water treatments and the conventional treatment were modeled using a general linear model (Minitab, Inc., State College, Pa.). The model was configured with the base 10 logarithm of the RLU score as the response. There were three terms in the model: treatment type, with four options (EO water at 10, 7.5, and 5 min, and the conventional treatment); sample location (liner, elbow, claw, etc.) repetition number to ascertain the effect of the multiple repetitions; and a binary variable to code whether the sample was taken before (0) or after (1) cleaning. Tukey's comparison of samples before and after cleaning by treatment type was used to attempt to discern differences at the 95% confidence level.

Results and Discussion

Short-Term Evaluation

The data for both microbial enrichment and ATP bioluminescence testing are shown in table 1. For the microbial enrichment cultures, there were very few "before cleaning" swabs that tested negative and very few "after cleaning" swabs that tested positive. Across all EO water treatments and repetitions, there were only five enrichment cultures that exhibited growth out of a total of 72 post-treatment swabs. There was no discernable trend to the positives, which could have been the result of contamination while collecting the samples. Therefore, considering only the microbiological data, it would be impossible to determine differences between any of the treatments.

Table 1. Short-term evaluation data.

Treatment and Location	ATP Initial (RLU)	Enrichment Initial [a]	ATP Final (RLU)	Enrichment Final [a]
Conventional				
Claw	9177	3/3	6757	1/3
Elbow	18092	4/6	756	1/6
Liner	15162	6/6	1395	0/6
Pipe	13869	4/6	979	2/6
Receiver	9868	2/3	0	0/3
EO (10 min)				
Claw	6611	3/3	114	1/6
Elbow	15196	6/6	0	2/6
Liner	16148	6/6	776	1/6
Pipe	14111	6/6	0	0/6
Receiver	7359	3/3	0	0/3
EO (7.5 min)				
Claw	27767	3/3	53	0/3
Elbow	33175	4/6	0	1/6
Liner	46715	5/6	1644	1/6
Pipe	28849	5/6	480	0/6

Receiver	16801	2/3	0	0/3
EO (5 min)				
Claw	24703	3/3	2581	0/3
Elbow	31312	6/6	2757	0/6
Liner	16518	6/6	4241	0/6
Pipe	25790	6/6	3362	0/6
Receiver	25071	2/3	2869	0/3

[a] Results for enrichment cultures are displayed as a fraction of the total number of samples across all repetitions that resulted in a positive growth in the enrichment culture.

The RLU scores from the ATP bioluminescence analysis are also summarized in table 1. Note that the "before treatment" RLU scores range from below 10,000 to nearly 50,000, which might be explained by the variability of the swab sampling method. This also seems consistent with the manufacturer's recommendation against using the device as a quantitative measurement of soil. A simple, non-statistical observation from the "after treatment" RLU scores indicates that the 10 min EO water treatment removed most or all of the soil from the non-porous surfaces, and seems to have cleaned the system better than the 5 min EO water treatment or even the conventional treatment. The statistical analysis was used to evaluate these differences and determine if they are statistically significant.

Statistical Analysis of the Short-Term Evaluation

The magnitude of error increases as the level of soil increases, so the data were normalized by transforming them with a base 10 logarithm. Log $_{10}$ transforms are commonly used in microbiology and sanitation. Because log $_{10}$ (0) is undefined, log $_{10}$ (0) values were replaced with zero so that all measurements were defined.

Because of the number of factors involved, the general linear model procedure was used in Minitab to model the data. Treatment type, location type of each sample, and repetition number (1, 2, or 3) were all used to model the log $_{10}$ (RLU) data. In addition, a binary code variable "cleaned" was added to the model to differentiate the initial condition samples from the post-treatment samples. The "cleaned" variable was nested within treatment so that post-treatment RLU scores are compared against initial RLU scores from the same treatment. By modeling the data using a general linear model, comparisons can be made between the different treatments while accounting for variability caused by swabbing location or repetition number.

The ANOVA model shows that the treatment type, the location type sampled, and the binary indicator variable (before/after cleaning) are all highly significant (table 2). The repetition number is not significant until the 83% confidence level. The marginally significant effect of repetition may be explained by the methodology: the system was subjected to a strong cleaning only prior to each set of treatments, not prior to each repetition. Therefore, each replicate might have been influenced by the results of the previous replicate, especially if the treatment did not completely clean the milking system.

Table 2. Short-term data ANOVA table for $\log_{10}$ (RLU).

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Treatment	3	23.936	23.936	7.979	7.06	0.000
Trial (treatment)	8	13.330	13.330	1.666	1.48	0.169
Cleaned (treatment)	4	450.036	450.036	112.509	99.62	0.000
Type	4	14.261	14.261	3.565	3.16	0.016
Error	172	194.249	194.249	1.129		
Total	191	695.813				

Several comparisons were made using Tukey's multiple comparison procedure (table 3). First, a comparison of the soiling levels before cleaning was evaluated. Next, the pre-cleaning RLU scores for each of the EO water treatments and the conventional treatments were compared with the after-cleaning RLU scores for the same treatment, showing that for each treatment a significant level of cleaning took place. Finally, the EO water treatments were compared with each other and with the conventional treatment.

Table 3. Tukey's comparison of $\log_{10}$ (RLU score).

Comparison	Difference of Means	SE of Difference	t-ratio	Adjusted P-Value
EO initial (10 min) - Conventional initial	-0.056	0.3068	-0.18	1.0000

EO initial (7.5 min) - Conventional initial	0.320	0.3068	1.04	0.9668
EO initial (5 min) - Conventional initial	0.150	0.3068	0.49	0.9997
Conventional final - Conventional initial	-2.783	0.3068	-9.07	0.0000
EO final - EO initial (10 min)	-3.557	0.3068	-11.59	0.0000
EO final - EO initial (7.5 min)	-3.620	0.3068	-11.80	0.0000
EO final - EO initial (5 min)	-2.000	0.3068	-6.52	0.0000
EO final (10 min) - Conventional final	-0.830	0.3068	-2.704	0.1285
EO final (7.5 min) - Conventional final	-0.516	0.3068	-1.683	0.6979
EO final (5 min) - Conventional final	0.933	0.3068	3.041	0.0541
EO (7.5 min) - EO (10 min)	0.313	0.3068	1.020	0.9708
EO (5 min) - EO (10 min)	1.762	0.3068	5.744	0.0000
EO (7.5 min) - EO (5 min)	-1.449	0.3068	-4.724	0.0001

Comparison of Equality of Soiling

To verify that the milking system was soiled at the same level before each treatment, Tukey's comparison was used to compare the pretreatment conditions for each EO water treatment with the pretreatment conditions for the conventional treatment. According to the analysis, there were no significant differences between the "before cleaning" RLU scores for the conventional treatment and the "before cleaning" RLU scores for any EO water treatment.

Comparison of Soiled Versus Clean

The next comparison shows that there were significant differences between the initial condition of the system and the condition of the system after cleaning. Without significant differences, it would be impossible to conclude that any cleaning took place. All of the differences were significant at the 95% confidence level, meaning that a significant removal of soil occurred during the cleaning at all EO water treatment levels as well as with the conventional treatment.

Comparison of EO Water and Conventional Treatments

Finally, a comparison of the post-treatment conditions between and among each EO water treatment and the conventional treatment indicates the relative effectiveness of each treatment. The comparison between the RLU scores of the cleaned systems showed that there were no significant differences at the 95% confidence level between the conventional treatment and either the 10 min or 7.5 min EO water treatments. The system was significantly less clean with the 5 min treatment than with the conventional treatment, and significantly less clean than with either the 10 min or 7.5 min EO water treatments. Although not significant at the 95% level, the comparison between the conventional treatment and the 10 min EO water treatment would be significant at an 87% level ($P = 0.13$). However, if these differences were interpreted as significant differences, then the difference shows that the 10 min EO water treatment resulted in lower RLU scores than the conventional treatment. There were no significant differences between the 10 min and 7.5 min EO water treatments.

Long-Term Evaluation

The goal of the long-term evaluation was to determine if cleaning problems might occur during the long-term use of EO water. Since there were no significant differences between the 10 min EO water treatment and the 7.5 min EO water treatment for the short-term evaluation, the 7.5 min treatment was selected for the long-term evaluation.

In general, the milking system does not appear to have been sufficiently cleaned by the 7.5 min EO water treatment (table 4). All of the average RLU scores for EO water treatment are higher than the corresponding scores for the conventional treatment, although the conventional treatment RLU scores are also above zero, indicating a poorly cleaned milking system.

Table 4. Long-term evaluation: 7.5 min EO water treatment.

	Trial 1		Trial 2		Trial 3	
Swab Location	ATP (RLU)	Enrichment Culture [a]	ATP (RLU)	Enrichment Culture [a]	ATP (RLU)	Enrichment Culture [a]
Claw	9968	+	115304	+	0	-

	20770	N/A	269000	N/A	3306	N/A
Elbow	0	-	0	-	2093	-
	263	-	6802	-	743	-
	0	N/A	5625	N/A	2406	N/A
	0	N/A	7150	N/A	4275	N/A
Liner	0	-	1515	-	2534	-
	29	-	1277	-	57150	-
	602	N/A	470	N/A	1847	N/A
	0	N/A	843	N/A	5820	N/A
Pipe	0	-	4300	+	0	-
	388	-	4459	-	1254	-
	0	N/A	0	N/A	N/A	N/A
	0	N/A	8734	N/A	N/A	N/A
Receiver	14931	-	0	-	0	-

[a] For the enrichment cultures, a "+" indicates growth in the enrichment culture, and a "-" indicates no growth in the enrichment culture. Claw assembly was hand washed prior to trial 3.

Looking beyond the average scores, the raw RLU scores furnish a more detailed view of the results. For the first replication, the 7.5 min EO water treatment cleaned the milking system comparatively well, with only a few non-zero readings on the stainless steel surfaces, and even the majority of the rubber liners registered an RLU score of zero or near zero (less than 100). The polysulfone claw had the highest RLU score in this replication, and by the end of the second replication there was a visible white film with a greasy texture on the claw and corresponding RLU scores of above 100,000. The other materials in the milking system also exhibited high RLU scores, although a visible film was not apparent. The visible film on the claw remained after the strong cleaning, so the claw was disassembled and scrubbed with a towel

in a sink filled with conventional alkaline dairy wash before the third replication. After the third replication, the claw was clean and attained an RLU score of zero for one of the two measurements. Results for the enrichment cultures are also listed in table 4. As in the short-term experiment, most of the enrichment cultures did not detect the presence of bacteria. There was only one location, the claw, where the majority of the enrichment cultures (2 out of 3) were positive. It seems likely that the trial 3 enrichment culture would have been positive had the claw not been scrubbed by hand before the third replication.

The results for the conventional treatment were also mixed (table 5). The first replication resulted in mostly non-zero and therefore unacceptable RLU scores. However, in the second replication, nearly all of the RLU scores were zero. The enrichment cultures were mostly negative, with only three swabs testing positive out of 16. A third replication with conventional chemicals was not attempted. Since the EO water treatment obviously performed poorly, there was little to be gained by replicating the conventional treatment.

Table 5. Long-term evaluation: Conventional treatment.

Swab	Trial 1		Trial 2	
Location	RLU	Enrichment	RLU	Enrichment
Claw	34390	+	336	-
	23929	N/A	0	N/A
Elbow	0	-	2140	-
	552	-	0	-
	397	N/A	0	N/A
	3479	N/A	0	N/A
Liner	6068	-	0	-
	8004	-	0	-
	4702	N/A	0	N/A
	2779	N/A	0	N/A

Pipe	786	+	0	+
	954	-	0	-
Receiver	0	-	12275	-

Statistical Analysis of the Long-Term Evaluation

Before fitting the ANOVA model, the RLU scores were transformed using the base 10 logarithm, substituting "0" for any $\log_{10}(0)$ values. The model had the same factors as the short-term evaluation, except that the binary indicator variable ("cleaned") was not necessary because the long-term test data set contained only post-treatment evaluations.

According to the ANOVA model, the factor "treatment" ($P = 0.189$) was not a significant source of variance. Therefore, any comparisons between the treatments would not show significant differences. The factor indicating the trial number was highly significant, corroborating the general observation that there were large variations between the treatment replications.

Conclusion

In the short-term evaluation, there were no significant differences in cleanliness, as measured by ATP bioluminescence swabs, between the full-scale pipeline milking system when cleaned with the EO water treatments (7.5 and 10 min) and when cleaned with the conventional treatment. Therefore, the EO water treatment is as effective at cleaning the pipeline milking system as the representative conventional treatment.

The long-term study used only the 7.5 min EO water treatment and did not appear to reliably clean the system based on ATP bioluminescence data. Therefore, a 7.5 min EO water treatment during the long-term study did not clean the pipeline milking system when applied on a repeated basis. Because longer EO water treatments were not attempted, it is possible that the 7.5 min treatment time was simply too short to effectively clean the system on a repeated basis. Based on the results of the short-term study, electrolyzed oxidizing water shows potential as an effective cleaning agent for milking systems. Future research should include a long-term evaluation of 10 min or longer EO water treatments.

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