

DISINFECTION IN A DAIRY MILKING PARLOUR USING ANOLYTE AS DISINFECTION

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INTRODUCTION

Dairy products such as milk, butter, cream and cheese are all susceptible to microbial spoilage because of their chemical composition. Milk is an excellent growth medium for all of the common spoilage organisms, including molds and yeasts. Fresh, non-pasteurized milk generally contains varying numbers of microorganisms, depending on the care employed in milking, cleaning, and handling of milk utensils. Raw milk held at refrigerator temperatures for several days invariably shows the presence of several or all bacteria of the following genera: *Enterococcus*, *Lactococcus*, *Streptococcus*, *Leuconostoc*, *Lactobacillus*, *Microbacterium*, *Propionibacterium*, *Micrococcus*, coliforms, *Proteus*, *Pseudomonas*, *Bacillus*, and others. Those unable to grow at the usual low temperature of holding tend to be present in very low numbers. The pasteurization process eliminates all but thermotolerant strains, primarily streptococci and lactobacilli, and spore formers of the genus *Bacillus* (and clostridia if present in raw milk). The spoilage of pasteurized milk is caused by the growth of heat-resistant streptococci utilizing lactose to produce lactic acid, which depresses the pH to a point (about pH 4.5) where curdling takes place. If present, lactobacilli are able to grow at pH values below that required by *Lactococcus lactis*. These organisms continue the fermentative activities and may bring the pH to 4.0 or below. If mold spores are present, these organisms begin to grow at the surface of the sour milk and raise the pH toward neutrality, thus allowing the more proteolytic bacteria such as *Pseudomonas* spp. to grow and bring about the liquefaction of the milk curd.

The use of a well balanced cleaning and sanitizing programme will aid in the production of raw milk of exceptionally high microbiological quality. Farms with well disciplined and carefully organized sanitation programmes which extend from cow preparation to bulk tank cleaning, are easily achieving Standard Plate Counts of <10 000/ml and coliforms counts of <10/ml for raw milk – standards usually not reached for fluid pasteurized milk by most processing plants in South Africa.

Chemical sanitizers are normally used and iodophors (25 ppm iodine) and chlorine (100 ppm chlorine) are the most widely used and allowed a minimum contact time of two minutes for best results.

A novel way of the electro chemical activation of water was recently introduced in South Africa. During Electro Chemical Activation (ECA) of water, a dilute saline solution is “activated” by passing through a cylindrical electrolytic cell in which the anodic and cathodic chambers are separated by a permeable membrane. Two separate streams of

activated water are produced: Anolyte with a pH range of 2-9 and an oxidation- reduction potential (ORP) of +400 mV to +1200 mV. Anolyte is an oxidizing agent due to a mixture of Free Radicals and has an antimicrobial effect. Catholyte with pH of 12 to 13 and an ORP of about -900mV, it has reducing and surfactant properties and is an antioxidant. During the process of electrochemical activation three broad classes of product are produced:

- Stable products – these are acids (in the Anolyte) and bases (in the Catholyte) which influence the pH of the solution in question, as well as other active species.
- Highly active unstable products – these include free radicals and other active ion species with a typical lifetime of less than 48 hours. Included here would be electrically and chemically active micro bubbles of electrolytic gas 0,2 – 0,5 micrometer in diameter and with concentrations up to 10^7 ml^{-1} , distributed uniformly through the solution. All these species serve to enhance the oxidation – reduction potentials (ORP) of the Anolyte.
- Quasi-stable structures – these are structures formed at or near the electrode surface as a consequence of the very high voltage drop (10^7 V cm^{-1}) in those regions. These are free structural complexes of hydrated membranes around ions, molecules, radicals and atoms. The size of these water clusters is reduced to approximately 5-6 molecules per cluster. All these features enhance the diffusion, catalytic and biocatalytic properties of the water.

The chemical composition of ECA solutions may be altered by utilizing various hydraulic arrangements linking electrolytic cell modules, together with other supplementary devices, in order to optimally address the requirements of specific areas of application. Some other variables are flow rate; hydraulic pressure; current density and voltage on the electrodes.

One of the most important problems for researchers into ECA processes' mechanism is that of the nature of the state metastable water and diluted water solutions find themselves in after unipolar electrochemical exposure. Until now this problem has not been satisfactorily solved, nevertheless it is not an obstacle to wide practical application of electrochemically activated liquids. The problem lies in the fact that it is extremely difficult to assess activation contribution of purely chemical and purely physical components of electrochemical effect on para-electrode environment.

During anode electrochemical treatment, water acidity grows. ORP increases due to the formation of stable and unstable acids (Sulfuric, hydrochloric, hypochlorous, persulfuric), as well as hydrogen peroxide, peroxy-sulfates, peroxy-carbonates, oxygen-containing chlorine compounds and different intermediate compounds arising in the process of spontaneous decomposition and interaction of the indicated substances. Also, as a result of anode electrochemical treatment surface tension somewhat decreases, electric conductivity rises, as does the content of dissolved chlorine and oxygen, concentration of hydrogen and nitrogen decreases, and water structure changes.

A range of bactericidal substances, commonly termed biocides or microbicides, are available, all of which are claimed by their agents to kill bacteria in aqueous systems quantitatively. However, different bacteria react differently to bactericides, either due to differing cell wall properties⁶, or to other mechanisms of resistance, either inherent or inducible^{7,8,9}.

The bacterial cell membrane provides the osmotic barrier for the cell and catalyses the active transport of substances into that cell. Alternations in transmembrane potential caused by the action of electron donor or electron acceptor factors are associated with powerful electro-osmotic processes accompanied by water diffusion against ORP gradients, with resultant rupture of the membranes and outflow of the bacterial cell contents. The bacterial membrane itself has an electrical charge. The anions present in Anolyte act on this membrane. Anolyte can also disrupt other functions of the cell. Unlike “higher” organisms, single celled organisms such as bacteria obtain their energy sources from the environment immediately outside the cell. Small molecules are transported across the cell membrane via an electro-chemical gradient. Thus, any significant change in the ORP of the immediate environment has drastic consequences for the cell. Even if instantaneous death of the cell does not occur, all enzymatic functions in the membrane are affected and this will also result in loss of cell viability.

MATERIALS AND METHODS

Three milking stations (same everyday) were analysed on a daily basis after disinfection. Four different surfaces on each of the three stations were sampled each day. The four different surfaces sampled were (1) the inside of the teat cluster, (2) teat cluster top (mouth) (3) float control flow sensor inside and (4) float control flow sensor lid. A sterile swab was used to sample each surface and streaked out on nutrient agar plates. The plates were incubated at 37°C for 48h and the number of colonies formed on each plate was counted.

Sporeformers detection

Cell suspensions were prepared from the plates that gave spreaders in the dairy disinfection experiment. The colonies were suspended by adding 5 ml of sterile distilled water to the plate and suspending the colony using a sterile loop. The suspensions were transferred to test tubes and the test tubes were incubated at 80°C for 10 min. 1 ml of each of the cell suspensions was plated out on nutrient agar plate. The plates were incubated at 37°C for 48h.

Bulk tanker washing

The milk tank was washed with the usual disinfectant (control) and thereafter the different surfaces inside the tank were sampled using Rodac plates. For the experiment, the tank was washed with ECA solutions. The first wash was done with catholyte, then the tank was rinsed with tap water and the tank was finally rinsed with anolyte. The inside of the tank was sampled as for the control. The experiments were run for five days

for each disinfectant used. The plates were incubated at 37°C for 24h then numbers of colony forming units were counted.

RESULTS AND DISCUSSION

Table 1. Microbiological analysis of different surfaces in a milking parlour after disinfection.

	Control (cfu per swab)										
	1	2	3	4	5	6	7	8	9	10	11
Station 1											
FSL	Spr	>300	0	>300	14	Spr	Spr	Spr	>300	Spr	>300
FSI	Spr	119	154	115	0	>300	>300	Spr	>300	Spr	246
TCI	0	3	0	7	0	2	0	>300	105	0	0
TCT	>300	Spr	>300	>300	Spr	>300	>300	>300	>300	>300	>300
Station 2											
FSL	Spr	>300	Spr	>300	Spr	Spr	Spr	Spr	>300	Spr	Spr
FSI	279	>300	>300	>300	Spr	>300	149	Spr	98	Spr	0
TCI	0	51	0	6	61	38	1	2	0	Spr	90
TCT	>300	Spr	>300	>300	6	Spr	>300	>300	>300	Spr	>300
Station 3											
FSL	3	>300	Spr	Spr	Spr	Spr	Spr	Spr	>300	Spr	Spr
FSI	>300	>300	>300	273	9	Spr	292	>300	>300	82	>300
TCI	0	6	186	6	0	0	1	Spr	1	3	182
TCT	>300	>300	>300	>300	Spr	>300	>300	Spr	>300	Spr	>300

Spr= spreader

cfu= colony forming units

FSL= float controlled flow sensor lid

FSI= float controlled flow sensor inside

TCI= teat cluster inside

TCT=teat cluster top

The results in Table 1 and Table 2 are qualitative rather than quantitative, since it was impossible to sample exactly the same surface area, due to the nature of the sampled surface. The normally used method of disinfection (indicated as the control in the results) did have some degree of microorganism control (**Table 1**). In most cases, the teat cluster inside had the lowest level of contamination. However, most of the surfaces were not satisfactorily disinfected, indicated by the spreaders (**Table 1**), and on some surfaces where number of microorganisms exceeded the maximum number that could be counted on the plates. The relatively higher numbers of microorganisms in the float control flow sensor lid and teat cluster top, was attributed to these surfaces not being exposed to the disinfectant solutions (**Table 1 and Table 2**). The anolyte however eliminated the spreaders on these sampling sites. When anolyte was used as a disinfectant, the results were generally better. This is particularly evident when comparing the results of the FSI sampling point (**Table 1 and Table 2**)

Table 2. Microbiological analysis of different surfaces in a milking parlour after disinfection with anolyte

	Anolyte(cfu/ml)					
	1	2	3	4	5	6
Station 1						
FSL	246	94	>300	0	69	0
FSI	1	0	0	1	0	0
TCI	0	0	0	1	0	4
TCT	>300	>300	0	>300	>300	>300
Station 2						
FSL	110	5	>300	>300	185	>300
FSI	0	0	1	0	1	0
TCI	0	0	2	0	0	9
TCT	>300	>300	44	78	>300	>300
Station 3						
FSL	69	>300	Spr	>300	6	0
FSI	3	0	Spr	0	0	0
TCI	0	13	0	0	4	9
TCT	>300	164	109	>300	>300	>300

Spr= spreader

cfu= colony forming units

FSL= float control flow sensor lid

FSI= float control flow sensor inside

TCI= teat cluster inside

TCT=teat cluster top

Table 3: Growth of cells after exposure to a high temperature

Plate number	Results
1 – 18	G

G=Growth

All the spreaders were resistant to heating at 80°C (**Table 3**). This indicated that all the spreaders were a result of growth of sporeforming organisms that contaminated the milking parlour.

Table 4: Results of the tanker after cleaning with control disinfectant and ECA solutions (Catholyte followed by Anolyte)

Plate no.:	Control					ECA solutions				
	Days									
	1	2	3	4	5	1	2	3	4	5
	cfu/cm ²									
1	Spr	288	TNTC	120	19	NG	39	NG	NG	NG
2	Spr	75	264	TNTC	2	8	9	NG	NG	NG
3	219	NG	TNTC	226	34	NG	12	33	NG	NG
4	202	126	108	258	26	44	12	Spr	NG	NG
5	TNTC	206	TNTC	Spr	6	NG	TNTC	NG	NG	NG

TNTC= Too numerous to count (> 300 cfu/ 25cm²)

NG = No growth

Spr= Spreader

The numbers of cfu/cm² were higher when the tank was washed with the control disinfectant, than when it was washed with ECA solutions throughout the experimental period (Table 4). The control disinfectant was not effective for disinfecting the bulk tank, since most of the counts were higher than 300 cfu/cm² and spreaders (sporeformers being encountered regularly (Table 4). Effective disinfection of the milk tank was however achieved using a Catholyte wash followed by an Anolyte disinfection with most surfaces being sterilized (Table 4).

CONCLUSIONS

- The contact between the disinfectant and the surface to be disinfected is essential for removing the number of organisms.
- All spreaders were identified as spore forming organisms
- Anolyte eliminated the spore forming bacteria
- Overall, the anolyte gave better disinfection than the control disinfectant

- Where anolyte made contact with a surface, disinfection was at an acceptable level, with most surfaces being sterilized
- The combination of Catholyte followed by an Anolyte disinfection step was effective for disinfection of the bulk tank.

REFERENCES

Jay J. M. (1992). *Modern Food Microbiology*. 4th edition. Chapman & Hall, New York. ISBN 0-442-00733-7.