SURFACE DISINFECTION, USING ANOLYTE

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1. INTRODUCTION

All dairies, abattoirs, breweries and food processing plants must be kept as clean and hygienic as possible. When dirty equipment is not in use, a rapid build-up of micro-organisms occurs which can result in severe contamination of the foodstuff when the equipment is re-used. If proper attention is not given to the use of clean equipment and reduction of contamination, the foodstuff will spoil rapidly. Proper sanitization will reduce the number of bacteria in all work areas and on equipment.

Sterilizing should not be confused with sanitizing or disinfecting. To sterilize means to destroy all forms of life applied especially to microorganisms, including bacterial and mould spores, and the inactivation of mould spores. There are no degrees of sterilization, an item is sterile or it is not.

Sanitizing is reducing the number of bacterial contaminants to levels judged safe by Public Health authorities. It implies a degree of physical cleanliness, i.e. the sanitizer is applied to a pre-cleaned surface.
To disinfect is to literally free from infection. This term has come to imply chemical treatment of an inanimate surface or substance to rid it of harmful micro-organisms. Disinfectants are frequently expected to perform their function in the presence of significant quantities of dirt and/or organic matter.

Sanitizing of equipment and utensils is best carried out just prior to use. It is a most important step in the general sanitation operation for the following reasons:

- A variety of micro-organisms may remain on food processing equipment after it has been washed, even though it may appear clean. The organisms my be types which have been slowly accumulating on the equipment or in the product during the processing operation. These can be removed, after cleaning the equipment, by thorough sanitizing.
- During the period that processing equipment is idle, large numbers of bacteria may develop even though the equipment was cleaned and sanitized. This especially true of surfaces which are difficult to dry. There are usually sufficient nutrients to support bacterial growth even on a clean surface and if it is moist, the increase in bacteria before the next usage may be tremendous.

- There may possibly be opportunity for insects or even rodents to contact idle equipment and this may result in appreciably contamination.
- Water supplies occasionally become contaminated and even Municipal after supplies are sometimes of questionable quality. When such water is employed for washing or rinsing equipment, spoilage organisms may contaminate the equipment. The use of a sanitizing agent in the water used to rinse equipment helps prevent such contamination.
- A programme of effective sanitizing can make an appreciable and measurable contribution to the quality and shelf life of food products.

1.1 Selection of sanitizers

There are many types of chemical compounds used in the formulation of disinfectants and sanitizers. However, in the food industry the number of varieties which can be used is severely limited for a number of reasons.

- The compounds must not be toxic to humans in as much as their residues on food must not be harmful in any way.
- They must not taint the product and must therefore be completely odourless.
- They must not colour the product in any way.
- They must be relatively safe to use for hand cleaning situations.
- Most essential is high bactericidal activity.

When selecting a sanitizer, the five most popular types should be considered for their respective merits. Known by their primary ingredients they are chlorine compounds, iodophores or iodine compounds, quaternary ammonium compounds (QATS), acid anionic surfactant germicides and hydrogen peroxide.

Chlorine based sanitizers are most widely used. Proven in use and acceptance over the years, they have excellent germicidal power against a wide range of bacteria. In properly blended products, they are relatively non-toxic at use concentrations (200 ppm), colourless, non-staining, easy to prepare and apply. Generally, they are also the most economical. Effective cleaning is essential when using these sanitizers as some of the available chlorine may be readily consumed by organic matter other than bacteria. Possible flavour problems associated with these products should be borne in mind in the brewing industry.

Chlorine is highly corrosive to a number of metals and its use is best confined to equipment fabricated in stainless steel. Temperature is another important parameter as the effectiveness of chlorine increases with increase in temperature. However, above 50°C the liberated chlorine is rapidly lost to the atmosphere, reducing the effectiveness of the solution.

Chlorine compounds should therefore not be used above 50°C neither should they be used where smoked products are being handled. This is because the phenolic

compounds in the smoke may react with the chlorine, producing chlorophenols which have a very strong odour.

Iodophores

The iodophores are basically a combination of iodine and a solubilizing agent that releases free iodine when diluted with water. They possess quick microbial action against a wide variety of microorganisms. At use concentrations, they are non-staining, relatively non toxic, non irritating and stable. No potable rinse is required if use concentration does not exceed 25 ppm available iodine.

Iodophores penetrate soil rapidly and are highly germicidal at virtually all concentrations. Many iodophores are approved for 'no rins' sanitizing applications at 25 ppm I_2. Iodophore use solution temperatures should not exceed 48°C or they will begin to 'gas-off'. The germicidal performance of different iodophore formulations may differ greatly. Products yielding the same pH and iodine concentration may yield vastly different germicidal activities at equivalent dilutions. Iodophores can be used in very hard water.

QATS

The quaternary ammonium compounds are types of cationic detergents processing good antibacterial activity. Unfortunately their detergency properties are very poor, but they are good wetting agents. They are widely used throughout the food and meat industries and commonly formulated with detergents to form detergent/sanitizers, which clean and kill bacteria in one operation. They can also be used on their own. Although extremely effective for killing a wide spectrum of bacteria, some groups are resistant to them. In use concentrations (200ppm) QATS are odourless, colourless and non toxic. They are stable when heat and in presence of organic soil. No potable water rinse is required if concentration is at or below 200ppm active ingredient. They should not be used on processing equipment in a brewery because of possible adverse effects on head retention and flavour.

The bacteriostatic properties of the QATS plus their stability and the property of being absorbed onto surfaces results in such remaining sanitized for many hours after treatment.

QATS have generally been applied in preference to chlorine under conditions of heavy organic contamination where, to overcome the presence of the organic material, the strength of the chlorine would have to assume corrosive proportions. Generally, they are combined with specific non-ionic detergents for sanitizing dairy equipment.

QATS can be adversely affected by water hardness and may be incompatible with other compounds. They are completely inactivated by anionic compounds such as

soaps. Acidity decreases the efficiency of many QATS to such an extent that as pH 3 their germicidal activities almost disappear while at pH 10, they show greatly improved activity. Temperature also affects their activity and an increase of about 20°C normally doubles it.

Acid anionics

Acid anionics surfactant germicides are combinations of organic or inorganic acids with surface active agents. The acid is usually phosphoric. The germicidal effect is provided by the low pH as well as the activity of the surfactant. The acidity of this type of germicide is effective in removing or controlling the formation of mineral films. Acid anionics are low foaming and are ideal for use in C.I.P. systems They are effective in hard and soft water and eliminated the need for acid rinsing. They are also non-corrosive to stainless steel.

Peroxide

Hydrogen peroxide containing sanitizers can be used in dairies, breweries and food processing plants. Using this sanitizing method does away with many of the disadvantages held by other sanitizers. Hydrogen peroxide containing sanitizers supersede conventional halogen sanitizers (chlorine, iodine, etc.) and cause the disinfection action to be rapid. They are not detrimental to the environment as when hydrogen peroxide decomposes, hydrogen and oxygen are formed. It is a broad spectrum, fast acting sanitizer with extremely low toxicity.

Phenols

Phenolic based disinfectants should not be used inside food processing plants, as they have a strong odour which will contaminate foods. They have good cleaning and disinfecting properties and should be used in stables, poultry growing houses, toilets, drains and compounds. They should be used when diluted with warm preferably hot water. Hey also have good deodorising properties.

All cleaning and disinfecting chemicals should be used in concentrations recommended by the manufacturers. The temperatures at which they are used should also be checked.

Certain chemicals, when mixed with others with which they are not compatible may liberate dangerously toxic gases and vapours. For example, acid compounds should never be mixed with strong alkaline or caustic compounds. Serious burns or even death may result.

All disinfectant/sanitizers have a recommended contact time. This is the time required for them to kill the majority of bacteria they come into contact with, before manufacturing operations can begin again. As these times may vary from product to product, the manufacturers instructions must be followed.

Some do's and don'ts with sanitizers:

Do:
1. Take the time to measure the sanitizer correctly.
2. Add the sanitizer to the correct amount of water to make the correct solution for use.
3. Use a clean, dry container or bucket for the solution.
4. Wash away all dirt before using the sanitizer.
5. Discard the solution when the day's work is finished.

Don't
1. Use a sanitizer for sterilization.
2. Store instruments or cleaning tools in a sanitizer solution.
3. Top up sanitizer solution.
4. Use yesterday's sanitizer solution, make up a fresh one each day.
5. Mix sanitizers and detergents it may inactivate both.

A large number of cleaning and sanitizing chemicals are available under a wide variety of trade names. Many are claimed to be particularly suited for a specific industry, but the ultimate test of effectiveness is performance under working conditions. Expensive chemicals are often no more effective than properly used cheaper ones.

The problem of obtaining a representative sample of a foodstuff for examination is often difficult, and the microbiological assessment of surfaces is no less a problem, particularly where the spread of surface contamination is uneven and the surface rough, as is the case with animal carcases. Microbiologists have been concerned with the detection and enumeration of microorganisms on surfaces for over 50 years. The problem is a complicated one and even using the very best available techniques only a proportion of the bacteria or other microorganisms will be recovered, and sometimes this proportion is exceedingly small. A brief overview of surface sampling methods will follow surface

1.2 Surface sampling techniques

Literature dealing with the microbiological sampling methods for surfaces have been reviewed by Favero *et al.* (1968). These workers described four basic methods for enumeration of bacteria on surfaces, viz. (1) the swab-rinse, (2) the rinse, (3) the agar contact, (4) the direct surface agar plating. Some of these methods have more application than others in the food industry, and there are many variations of the basic types.

The swab-rinse

This has many forms, and is possibly the most widely used method. Essentially, a sterile swab is rubbed over the surface of the object to be sampled (the swab is moistened with sterile fluid if the surface is dry) and then the tip of the swab is broken into tube containing a sterile diluent, shaken, and the rinse fluid plated

with or on to an appropriate culture medium. There is often poor recovery of bacteria from the surfaces sampled, either because of the nature of the surface, or the amount of pressure applied to the swab, or the time and the speed of application to the surface. Different people use swabs in different ways, so the results may not be reproducible between samples, or between laboratories. The cotton also retains some of the microorganisms, causing reduced counts.

Various modifications have been made to reduce these errors. A sterile metal template can be used to outline a known area, inside which the swabbing is done. The time of swabbing can be standardized, e.g. 15 sec, and also the size of the swab and the amount and type of material used to make the swab. Likewise replicate swabs are sometimes used on the same area. Another approach to aid the recovery of bacteria from the swabs is to use a known weighed quantity of calcium alginate wool to replace the cotton wool and the alginate can be dissolved in Ringer's solution containing 1% of sodium hexametaphosphate. Higgins (1950) stated that calcium alginate swabs will dissolve in most sodium salts to give the soluble sodium alginate, thus freeing all the organisms taken up on the swab and giving a more accurate quantitative recovery. A special alginate wool was specified, free from the bactericidal action possessed by alginate containing a quaternary ammonium compound used in textile processing.

However, there is some evidence that the alginate swab does not recover small numbers of organisms as well as cotton wool (Barnes, 1952), and a suggestion that the alginate or sodium hexametaphosphate may be inhibitory to some micro-organisms (Angelotti *et al*., 1958; Strong, Woodburn & Mancini, 1961). Others have reported higher recoveries with the alginate (Higgins, 1950; Tredinnick & Tucker, 1951; Cain & Steele, 1953; Walter, 1955).

The rinse method

With this method the contaminated surface is either immersed (as in the case of small objects) in a sterile fluid, or the fluid brought into contact with the surface being examined. This may require asetic removal of part of the surface into the diluent. Though essentially more accurate since all the surface, or a known section of it, is being sampled directly by the diluent fluid, there are certain disadvantages. Recovery from the surface may be low if the surface is such that it tends to retain the bacteria. Thus, with poultry skin, recovery by this method is low unless special precautions are taken to remove the bacteria, e.g. by shaking an area of skin with an abrasive material such as rough sand in the sterile diluent.

Greasy surfaces are also difficult to sample by this method, and large objects or large areas of surfaces mean that only a small proportion can be sampled, and the results may not be representative of the whole due to uneven spread of contamination.

Agar contact methods

Although there are many modifications of the agar contact method, basically it involves pressing a sterile nutrient agar surface against the surface to be sampled. The agar is then incubated and the adhering microorganisms enumerated. By the very nature of the method, it is most useful for smooth flat surfaces, and since dilution is not possible, only small numbers of contaminants can be enumerated. Rough surfaces, heavily contaminated, or those contaminated by spreading bacteria or moulds are not suited to the method, if enumeration is required.

The agar sausage technique often gives a lower apparent recovery of microorganisms than is achieved by other sampling methods. The reasons for this have been discussed by Riddle (1967) who pointed out that since the organisms contaminating a surface are present as micro-colonies, the swabbing technique breaks these up and gives a measure of individual viable cells. The agar sausage method gives a mirror-image of the number and distribution of these loci of infection on the surface and these may consists of one or many microorganisms.

Pictorial methods of representing the results are useful when dealing with non-scientific staff. Such methods (cited by Riddle, 1967) are those of Hansen (1962), Ten Cate (1965), Buchli (1965), Van Schothorst, Mossel & Kempelmacher (1966) and Mossell, Kampelmacher & Van Noorle Jansen (1966). For more accurate assessment of the amount and spread of surface contamination the method of Hansen (1962) can be used. This is a statistical procedure for measurement of bacterial surface contamination which can be applied to the agar sausage. The method consists of taking ten replicate agar sausage impressions of a surface and plotting the colony counts graphically on probability paper so that the logarithmic mean and standard deviation can be calculated.

Direct surface agar plating

Contaminants on surfaces can be detected *in situ* by the direct surface agar plate (DSAP) method (Angelotti & Foter, 1958; Angelotti *et al*., 1958), where sterile melted agar is poured on to the surface to be sampled and left to solidify under a sterile cover. After incubation the colonies at the interface are counted. Small items can be placed in a petri dish and covered with agar. This is mainly a laboratory technique since food plant surfaces are generally large, fixed, and cannot be incubated at a desired temperature. It is not applicable to dirty surfaces, since growth becomes confluent.

RODAC plates

Microbiological sampling of food contact surfaces is useful for determining the degree of cleaning and sanitizing. The RODAC method give an estimate of the microorganisms surviving the sanitation process and repeated use of these tests

provide information which the sanitarian can use to judge the sanitary quality of clean equipment and food contact surfaces and the effectiveness of the programme.

The replicate organism detecting and counting (RODAC) plate technique is a direct contact method for determining the microbiological quality of disinfected surfaces (Fig 1).

2. MATERIALS AND METHODS

2.1 Laboratory evaluation of the efficacy of anolyte using *Eschrichia coli* as test organism.

2.1.1 Test organism,

An *Escherichia coli* suspension containing ca. 10^6 cfu/ml was used to determine the efficacy of different anolyte concentrations.

2.1.2 Anolyte concentrations

The following anolyte concentrations were used, 1:10; 1:25; 1:50; 1:75 and 1:100. Anolyte produced in the University of Pretoria laboratory was compared to anolyte produced by Radical Waters.

2.1.3 Experimental procedure

E.coli (10^6 cfu/ml) was added to the different anolyte solutions (paragraph 2.1.2). A total *E.coli* count was done before adition and again 5 min after exposure to the different anolyte concentrations.

2.2 Standard disinfection practise

The following standard method of cleaning and disinfection was followed:

Step 1 Rough down (removal of all gross soils eg. chickens, skin, chicken wings etc.) (Fig 2)

Step 2 Pre- rinse (Fig 3)

Step 3 Wash with detergent (Lift II, Ecolab) (Fig 4)

Step 4 Rinse detergent until clear. (Fig 5)

Step 5 Disinfect with a QAC sanitizer (poultry plant) and oxi-acid (dairy plant). (Fig 6)

Step 6 Rinse thoroughly (Fig 7)

When evaluating the anolyte as a surface disinfectant - Step 5 was replaced using different concentrations of anolyte (1:25 and 1:50 and 100 %).

2.3 Surface sampling technique

The RODAC plate technique was used for all surface samples (Fig 1). Nutrient Agar was used as culture medium. Samples were taken before and after disinfection on dry disinfected surfaces.

2.3.1 Surface disinfection rating system

Table 1 Rating system used during the study

Cfu/25 cm^2	**Rating**
0 - 20	5
20 - 50	4
50 - 100	3
100 - 150	2
150 - 200	1
>200	0

3. RESULTS AND DISCUSSION

3.1 Laboratory evaluation of anolyte as a disinfectant

Table 2 Total colony formation of *E.coli* after anolyte treatment (5 min exposure)

ANOLYTE DILUTIONS	**Cfu/ml**	
	UP Anolyte	Radical Waters anolyte
1:10	7 2 8 4	1 6 0 34

1:25	7 3 0 0	12 5 3 4
1:50	1 4 0 0	5 3 2 4
1:75	0 1 2 3	16 19 2 8
1:100	>300	>300
Control	$>10^6$	$>10^6$

All the anolyte dilutions, excepting the 1:100 dilution resulted in the effective killing of *E.coli* (Table 2). The anolyte produced in the laboratory unit compared favourably with the anolyte supplied by Radical Waters (Table 2). Based on these results, it as decided to use a 100 % anolyte solution a 1:25 and a 1:50 dilution during the surface disinfection trials.

3.2 Surface disinfection in a poultry processing plant using anolyte.

Table 3 Microbiological quality of surfaces in a poultry plant using the standard disinfection practise and different concentrations of anolyte.

Sample area	**Number of cfu/25 cm^2**				
	Control	**Standard**	**1:25**	**1:50**	**100%**
Fillet Belt					
1	>300	144 (2)	35 (4)	87 (3)	2 (5)
2	>300	99 (3)	59 (3)	71 (3)	4 (5)
3	>300	39 (4)	19 (5)	178 (1)	2 (5)
4	>300	34 (4)	57 (3)	68 (3)	8 (5)
5	>300	27 (4)	37 (4)	115 (2)	5 (5)
6	>300	12 (5)	43 (3)	55 (3)	ND -
7	>300	1 (5)	ND -	97 (3)	ND -
Rating		77 %	73 %	51 %	100 %
Fillet Table side					
1	>300	11	ND	ND	ND
2	>300	91	ND	ND	ND
3	>300	49	ND	ND	ND
4	>300	19	ND	ND	ND

5	>300	17	ND	ND	ND
6	>300	13	ND	ND	ND
7	>300	24	ND	ND	ND
8	>300	31	ND	ND	ND
Stainless Steel Table	>300	40 (4)	28 (4)	31 (4)	19 (5)
1	>300	36 (4)	1 (5)	76 (4)	0 (5)
2	>300	18 (5)	5 (5)		0 (5)
3	>300	18 (5)	9 (5)		0 (5)
4	>300	8 (5)	0 (5)		0 (5)
5	>300	3 (5)	7 (5)		0 (5)
6	>300	2 (5)	26 (4)		0 (5)
7	>300	1 (5)	14 (5)		0 (5)
8					
Rating		95 %	95 %	80 %	100 %

ND = Not determined due to the fact that this is not a food contact surface.
cfu = colony forming units
Values in brackets indicate the rating using the system in Table 1

The microbiological quality of non-disinfected surfaces (control), as expected exceeded 300 cfu/ml (Table 3)

The best disinfection (100 %) on the fillet belt was achieved with the neat anolyte solution, followed by the standard disinfectant (QAC at 77 %), the 1:25 anolyte dilution (73 %) and 1:50 anolyte dilution (50 %) (Table 3).

The best disinfection on the stainless steel tables was achieved with the neat anolyte solution (100 %) followed by the 1:25 anolyte solution (95 %) and the standard (QAC - 95 %) and then the 1:50 anolyte dilution (80 %).

The standard of disinfection achieved on the stainless steel surfaces was higher than on the fillet belt (Table 2 and 3). This was ascribed to the non-porous nature of the stainless steel compared to the more porous nature of the belt, making disinfection an easier task.

The 1:25 anolyte dilution gave a comparable result, compared with the standard disinfectant in all instances, where as the 1:50 dilution gave a markebly lower result. For surface disinfection in poultry processing it is therefore recommended that either a neat (100 %) anolyte solution be used for disinfection or a dilution no more than 1:25.

4. DAIRY BULK TANK DISINFECTION

The anolyte solution gave a 93 % disinfection efficiency rating, compared to 80 % using the standard disinfection practise (Table 4).

4.1 Surface disinfection in a milking parlour using anolyte as disinfectant.

Table 4 Microbiological quality of surfaces in a dairy plant bulk tank using the standard disinfection practise and anolyte

Sample	**Cfu/25 cm^2**	
	Standard	**Anolyte**
1	48 (3)	44 (3)
2	130 (2)	5 (5)
3	21 (4)	5 (5)
4	12 (5)	3 (5)
5	3 (5)	3 (5)
6	3 (5)	2 (5)
Rating	80 %	93 %

cfu = colony forming units
Values in brackets indicate the rating using the system in Table 1

5. CONCLUSIONS

5.1 All dilutions, excepting the 1:100 solution was effective for disinfection purposes using *E.coli* as test organism.

5.2 Anolyte proved to be an excellent surface disinfectant in a poultry processing plant, at concentrations of 1:25 and 100 %.

5.3 Anolyte proved to be an excellent surface disinfectant in the dairy application.

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