BACTERICIDAL ACTIVITY OF ELECTROLYZED OXIDIZING WATER ON FOOD PROCESSING SURFACES

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ABSTRACT

The efficacy of electrolyzed oxidizing water (EOW) as a disinfectant of food processing surfaces was evaluated. Bacterial inactivation was first tested by direct contact of EOW with bacterial suspensions. Then three different surfaces (teflon, stainless steel and ceramic) were artificially contaminated and bacterial survival was determined after treatment with water and EOW. Efficacy was tested for mesophilic bacteria and for *Salmonella Typhimurium, Listeria monocytogenes*, verotoxigenic *Escherichia coli* O157:H7 and *Staphylococcus aureus*. Contact test (30 s) of EOW with bacterial suspensions resulted in an 8 log reduction in bacterial populations. Spraying treatment of artificially contaminated surfaces revealed different degrees of disinfectant activity of EOW on different bacterial species. No differences in efficacy were detected between different surfaces. Changes in the physico-chemical properties (pH, oxidation-reduction activity (ORP), free CHLC) of EOW were measured during contact with different surfaces. After low-pressure spraying both ORP activity and free-chlorine content rapidly decreased with time. Low-pressure spraying does not seem to affect EOW disinfectant activity. EOW has a very strong disinfectant activity which, along with its easy and safe use, makes it a good alternative to many other more widely used disinfectants.

- Key words: bacterial inactivation, electrolyzed oxidizing water, food, surfaces -

INTRODUCTION

Problems related to food safety are a major public health issue. Foodborne pathogens are currently estimated to be responsible for one-third of human diseases in the developed world. Although animals and humans are a major source of bacterial food contamination, indirect contamination caused by processing plant environment and production equipment plays a significant role. Disinfecting food processing surfaces is a key procedure for reducing and even eliminating bacterial contamination which might cause changes to the sensory characteristics of foodstuffs or be responsible for foodborne disease outbreaks (PARK *et al.*, 2002). Cross contamination via inanimate surfaces is an important factor in food-borne infections (DEZA *et al.*, 2005) and a wide variety of surfaces such as plastic, ceramic, stainless steel, glass, etc. can carry pathogenic microorganisms. Equipment in food processing plants, as well as protective clothing and gloves worn during working activities, have been shown to be carriers of foodborne pathogens (LIU and SU, 2006). Moreover, surfaces in public areas (toilets, laboratories, hospital instruments) carry high levels of bacteria and pathogens (PARK *et al.*, 2002). Procedures to reduce or even eliminate pathogens from surfaces is one of the key points of an effective HACCP program in the food industry and in controlling food contamination in homes, food markets, restaurants, health facilities and public areas (VENKITAANARAYANAN *et al.*, 1999).

Electrolyzed oxidizing water is obtained from the electrolysis of a NaCl solution (ca 2 g/L). When electricity flows through the NaCl solution it generates two types of water: the cathode produces alkaline electrolyzed water containing sodium hydroxide (pH 116, ORP ≈ -795 mV), while the anode produces acidic electrolyzed water containing hypochlorous acid (pH 2.4-2.7, ORP ≈ 1,150 mV); the concentration of the residual chlorine depends on the EO water machine setting.

The disinfectant activity of EOW lies in its low pH, its ORP activity and the presence of free chlorine and hypochlorous acid (SHARMA and DEMIRCI, 2003; STEVENSON *et al.*, 2004). Hypochlorous acid inactivates the bacterial cell by oxidation of sulphydric components of the membrane and by inactivation of respiratory enzymes, inhibition of ATP production and restraint of transport systems of the bacterial cell (PARK *et al.*, 2002). The strong oxidation-reduction activity of the EOW sequesters the membrane electrodes, rendering them unstable and helping antibacterial compounds to enter the bacterial cell. The low EOW pH influences the permeability of the bacterial membrane, affecting metabolic and reproduction activities of microorganisms (FABRIZIO *et al.*, 2002; LIAO *et al.*, 2007). EOW possesses antimicrobial activity against a variety of microorganisms on crops, bacteria and fungi. In recent years, EOW has gained interest as a disinfectant used in agriculture, dentistry, medicine and the food industry (HUANG *et al.*, 2007). EOW is an effective antimicrobial agent for cut vegetables like lettuce, alfalfa seeds, sprouts, pears, apples, peaches, tomatoes and strawberry, for cutting boards (VENKITANARAYANAN *et al.*, 1999), poultry carcasses (FABRIZIO *et al.*, 2002; PARK *et al.*, 2002; HINTON *et al.*, 2007), eggs (RUSSELL, 2003) and food processing equipment: for various surface materials commonly found in food processing facilities (AYEBACH and HUNG, 2005), stainless steel and glass surfaces (DEZA *et al.*, 2005), surfaces in food service areas (GUENTZEL *et al.*, 2007) and on *Listeria monocytogenes* biofilms formed on stainless steel (AYEBACH *et al.*, 2005; KIM *et al.*, 2001), as a sanitizer for treating different surfaces (PARK *et al.*, 2002b) and for use in abattoirs (BACH *et al.*, 2006), on seafood processing surfaces (LIU *et al.*, 2006) and gloves (LIU and SU, 2006) and for cleaning and disinfecting materials used in milking systems (WALKER *et al.*, 2005a, b).

The aim of this study was to evaluate the efficacy of electrolyzed oxidizing water (EOW) as a disinfectant for food processing surfaces to define its possible use in operating conditions in a slaughterhouse. The antibacterial characteristics of EOW and its changes with time after use were also tested to define the most appropriate ways of application.

MATERIALS AND METHODS

All tests were performed at the experimental slaughterhouse of the Veterinary Medicine Faculty of the University of Bologna (Italy).

EOW Solution

Electrolyzed oxidizing water was generated with a Vn 1000M EO water generator (Akuatech S.r.l® Treviso, Italy) through electrolysis of a dilute salt solution (3% NaCl). The current passing through the EO water generator was 90 amperes, the voltage between the electrodes was 24 volts and the flow rate was about 12 L/min. The EO water was used within a week after being produced. Chemical characteristics, like pH and oxidation-reduction activity (ORP), were measured on arrival at the laboratory with a pH metre (HI 98240, Hanna Instrument, Padova, Italy), using R334 and FC201D electrodes to measure ORP and pH, respectively. The direct iodometric titration method was used to determine free chlorine in the EOW solution (ISO7393-3:1990).

The principle of direct iodometric titration is the oxidation reaction under acidic conditions (pH 4 or less) of potassium iodide by chlorine with the release of free iodine: one mL of a saturated solution of potassium iodide was added

to a known volume of EOW solution. The free iodine was immediately titrated with a sodium thiosulfate standard solution until the yellow colour was almost discharged. Then 1 mL of a starch solution was added as indicator and the titration continued until the blue colour was completely discharged.

The concentration of free chlorine was then calculated by the volume of thiosulfate solution consumed.

Bacterial cultures

Two bacterial cultures were used to test the EOW solution: mesophilic bacterial suspension (MBS) and pathogen suspensions (i.e. *Salmonella Typhimurium, Staphylococcus aureus*, verotoxigenic *Escherichia coli* O157:H7 and *Listeria monocytogenes*).

The mesophilic bacterial suspension was obtained from samples collected at the experimental slaughterhouse of the Veterinary Medicine Faculty of the University of Bologna (Italy). After slaughtering activities and before cleaning and disinfecting the working areas, 100 cm² areas from three different types of surfaces (stainless steel viscera slip, teflon sectioning area and ceramic tile skinning area) were sampled with a sterile cotton swab. The collected material was suspended in 10 mL of saline solution and lat-

er 10 µL were plated on Petri dishes containing Standard Plate Count Agar (PCA, Oxoid) and incubated at 30°C for 72 h to allow the growth of the microorganisms. Bacterial growth was collected using a sterile plastic loop and then suspended in 100 mL of sterile saline solution. Turbidity was adjusted to obtain a bacterial suspension equal to number 3 on the MacFarland scale (about 9×10^8 CFU/mL). To determine the exact concentration of the bacterial suspension 100 µL of serial dilutions of the original mesophilic suspension were seeded and incubated in PCA Petri dishes at 30°C. A colony count was done after 72 h. The suspension was diluted once more before use.

Suspensions of the above-mentioned pathogens were also prepared. Detailed characteristics and origin of strains used in this study are listed in Table 1. Strains were seeded on Tryptone Soya Sgar (TSA, Oxoid, Cambridge, UK) supplemented with Bacto Yeast Extract (Difco, Maryland, USA) and incubated at 37°C. Bacterial growth was collected after 24 h and suspended in a 10 mL saline solution and turbidity was adjusted to number 3 on the MacFarland scale (about 9×10^8 CFU/mL). Each pathogen suspension used for the inactivation test was obtained by mixing equal parts of the prepared suspensions of the three available strains (Table 1). The exact titration of each pathogen

Table 1 - Bacterial strains used and their origin.

| Microorganism species	Strain	Origin
Salmonella Typhimurium	ATCC 14028	
29RA1	Wild strain from poultry	Isolated from infectious disease laboratory of the Department
6221/2155	Wild strain from pork	Isolated from infectious disease laboratory of the Department
Staphylococcus aureus	ATCC 25923	
270/5	Wild strain from cow's milk	Isolated from infectious disease laboratory of the Department
B/122/1	Wild strain from poultry	Isolated from food hygiene laboratory of the Department
Verotoxigenic E. coli O157: H7	ATCC 700927	
ED166	Wild strain from beef	Isolated from food hygiene laboratory of the Department
ED933	Wild strain from bovine faeces	Isolated from food hygiene laboratory of the Department
Listeria monocytogenes	ATCC 7644	
B/122/4	Wild strain from pork	Isolated from food hygiene laboratory of the Department
B/122/7	Wild strain from bovine faeces	Isolated from food hygiene laboratory of the Department
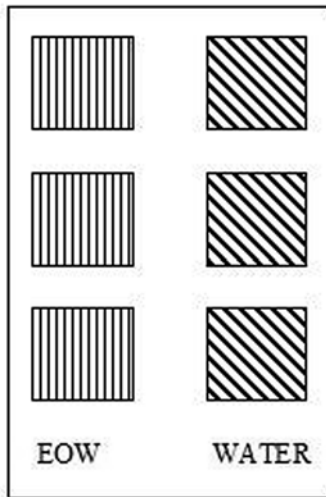
Fig. 1 - Artificially contaminated areas.

solution was checked by seeding 100 μL of serial dilutions of the initial suspensions in PCA Petri dishes and incubating them at 37°C for 24-48 h. All suspensions were diluted once more before use.

Test 1 or "contact test"

One mL of each bacterial suspension (mesophilic suspension, *Salmonella Typhimurium*, *Staphylococcus aureus*, verotoxigenic *Escherichia coli* O157:H7 and *Listeria monocytogenes*) was added to both 9 mL of acidic EOW and 9 mL of tap water. After incubation at room temperature for 30 s, 1 mL of each inoculated liquid was serially diluted using tubes containing 9 mL of neutralization buffer (Difco) and 0.L mL of each dilution was seeded in PCA Petri dishes to count mesophilic bacteria and each pathogen and then incubated at 30°C for 72 h and 37°C for 24-48 h, respectively.

To identify low numbers of bacteria or injured bacterial cells after treatment, 1 mL of the bacterial suspensions in contact with EOW (see above) was added to 20 mL of Tryptone Soya Broth supplemented with yeast (TSB-Y, Oxoid) and incubated at 37°C for 48 h to allow multiplication of bacteria. These broths (0.1 mL) were seeded in TSA-Y and incubated at 37°C. A colony count was done after 48 h of incubation.

Test 2: Efficacy on artificially contaminated surfaces

All tests were performed on equipment surfaces in the experimental slaughterhouse of the Veterinary Medicine Faculty of the University of Bologna (Italy) which are regularly used for slaughtering cattle, swine and goat-like animals. Tests were performed on three different surfaces: ceramic, stainless steel and teflon.

Six 100 cm² areas near one another were identified on the surfaces as illustrated in Fig 1. Be-

fore contamination, each area was disinfected by direct application of pure ethyl alcohol and then flamed with a portable Bunsen burner.

Contamination by mesophilic bacterial suspension (MBS): six 100 cm² areas were contaminated with 1 mL of a 10^7 CFU/mL suspension of MBS, for a total contamination surface area of about 10^5 CFU/cm².

Contamination by MBS and pathogen suspension: 5 mL of each pathogen suspension (*Salmonella Typhimurium*, *Staphylococcus aureus*, verotoxigenic *Escherichia coli* O157:H7 and *Listeria monocytogenes*) were mixed with 5 mL of MBS and diluted to obtain 10^7 CFU/mL suspensions. Each of these suspensions was used to contaminate six 100 cm² areas, that is, 1 mL of suspension was spread over each of the six 100 cm² areas.

After complete drying of the contaminated surfaces, treatment was performed by spraying tap water on three 100 cm² of the six contaminated areas for five seconds and spraying EOW on the remaining three areas for five seconds, using a low pressure pump with a 1.2 mm diameter outlet.

Once the surfaces had dried by unaffected evaporation to simulate the operating conditions, a sample was collected from each area and bacteria were isolated and counted. In detail, a sterile gauze was rubbed over the whole treated area 10 times and then put in a sterile plastic bag containing 100 mL of sterile saline solution. After mixing in a stomacher (Blender Eco 400, PBI, Milan, Italy) 1 mL of the sample obtained and 1 mL of five serial 10-fold dilutions were seeded in plastic Petri dishes containing PCA for mesophilic bacteria count (after incubation at 30°C for 72 h), and selective agar medium for each type of pathogen (37°C for 24-48 h).

Each of these experiments was repeated twice.

Test 3: EOW disinfectant activity after application on surfaces

In order to test the two materials with higher and lower conductivity, ORP, pH and free chlorine ion quantity, modifications were tested in EOW after contact with two different surface materials (teflon and stainless steel). EOW (200 mL) was placed in containers to form 2mL EOW films at the bottom of the containers. The physico-chemical characteristics above were measured after 0, 2, 4, 6 and 24 h of treatment.

The pH and ORP were measured with a Hanna Instrument HI 98240 pH metre with R334 and FC201D electrodes for measuring ORP and pH, respectively. Quantification of free chlorine ion was determined by iodometric titration following the procedure described by GARY (2003) and FABRIZIO *et al.* (2002).

Test 4: Influence of spraying on EOW chemical characteristics

EOW was sprayed in Teflon and stainless steel

containers by a low pressure pump with a 1.2 mm diameter outlet. Immediately after spraying, the liquid was collected and tested for pH and ORP.

RESULTS AND DISCUSSION

EOW solution

On delivery, EOW had a pH of 2.69, an ORP of 1,135 mV and the free chlorine concentration was about 110 mg/L.

Test 1 or "contact test"

The culture of tubes containing tap water had the same number of colonies as the amount inoculated (Table 2). No colony was isolated from the samples treated with EOW by direct plating. A statistically significant 8 log reduction ($p<0.05$) in the bacterial population was recorded comparing EOW with tap water.

Further enrichment of all samples confirmed total inactivation of bacterial cells in the tubes containing EOW.

Test 2: Efficacy on artificially contaminated surfaces

Table 3 shows the bacterial counts on each type of surface after treatment with either tap water or EOW. Significant differences ($p<0.001$) on bacterial inactivation were found between treatments with EOW and tap water for each microorganism under study. Complete inactivation was always obtained for *Salmonella Typhimurium*, verotoxigenic *Escherichia coli* and *Listeria monocytogenes*, especially after treatment with EOW. However, no differences were found when comparing the disinfectant activity on the three different surfaces.

Treatment with EOW for 5 seconds on artificially contaminated surfaces led to a significantly greater reduction of bacterial concentration than that obtained by treatment with tap water ($p<0.001$) for each microorganism. In detail, mesophilic bacteria were reduced by 2.28 log on the average, while pathogens showed an average

reduction of 1.77 log, with a maximum reduction of 2.12 log for *E. coli* O157:H7 and a minimum reduction for *S. Typhimurium* of 1.34 log.

No statistically significant difference in inactivation was found between the different surfaces for either mesophilic or pathogen bacteria. Only one test on ceramics showed an extremely high level of inactivation (3.23 log). However, the smooth nature of ceramics might be partly responsible for the greater efficacy of EOW. While stainless steel and teflon are more vulnerable to degradation with use and time, ceramics tend to maintain a smooth surface that facilitates activity of disinfectants against bacteria.

A major result was that complete inactivation of bacteria was reached by treatment with EOW only when the level of contamination after treatment with water was equal to or less than 2.1 log. This proves the higher efficacy of EOW compared to water and suggests that the final result may depend on initial contamination. Moreover, all tests were performed on artificially contaminated surfaces resulting in highly contaminated areas (10^5 CFU/cm^2). Future research should focus on the dynamics of inactivation at different initial bacterial concentrations.

The activity of EOW was compared with that of another disinfectant (iodophor, IOD), resulting in a greater efficacy of EOW especially in reducing total aerobic bacterial contamination of surfaces (BACH *et al.*, 2006).

Besides its greater efficacy as a disinfectant, other advantages of EOW compared with other well-known disinfectants include: i) no risk for users, ii) highly concentrated disinfectant solutions are avoided since EOW can be delivered ready to use or produced in the place of use (PARK *et al.*, 2002 a), iii) easy and relatively cheap to produce (FABRIZIO *et al.*, 2002) and iv) very low environmental impact (FABRIZIO *et al.*, 2002), whereas organic acid disinfectants may be very harmful to the environment and even modify the sensory characteristics of food products (FABRIZIO *et al.*, 2002).

Hypochlorite solution is one of the most widely used disinfectants, mainly because of its wide bactericidal spectrum and its relatively low price (LIU *et al.*, 2006). EOW has a higher bactericidal activity than an equally concentrated hypochlorite solution, mainly because of its low pH and high ORP (PARK *et al.*, 2002 a), and a 200 mg/L hypochlorite solution (LIU *et al.*, 2006; PARK *et al.*, 2005).

The bactericidal efficacy of EOW is reduced when relatively large amounts of organic material are present. This is characteristic of many other disinfectants, including hypochlorite (BACH *et al.*, 2006; LIU *et al.*, 2006). This effect can be reduced by using the alkaline solution derived from the production of EOW before applying the acidic EOW. The alkaline solution contains a high concentration of sodium hydroxide that acts as a detergent by reacting against fats and

Table 2 - EOW efficacy on bacterial suspensions.

Microorganism species	Bacterial population (log$_{10}$ CFU/mL)[a]	
	Tap water 30"	EOW 30"
Mesophilic bacteria	8.83±0.14	N.G.[b]
Salmonella Typhimurium	8.53±0.16	N.G.[b]
Staphylococcus aureus	8.89±0.01	N.G.[b]
Verotoxigenic E. coli O157:H7	8.60±0.09	N.G.[b]
Listeria monocytogenes	8.67±0.07	N.G.[b]

[a] average of six tests ± standard deviation;
[b] no growth of viable colonies in TSA Petri dishes.

Table 3 - Bacterial counts after treatment with either tap water or EOW on each type of surface.

Microorganism species	Bacterial population (log$_{10}$ CFU/ cm^2)[a]					
	Ceramics		Stainless steel		Teflon	
	Tap water	EOW	Tap water	EOW	Tap water	EOW
Mesophilic bacteria	2.77±0.09	0.9±0.4	2.31±0.15	0.15±0.27[c]	2.51±0.10	1.03±0.47
Salmonella Typhimurium	1.23±0.22	NG[b]	1.5±0.09	NG[b]	1.3±0.45	NG[b]
Staphylococcus aureus	2.30±0.06	0.38±0.40[c]	2.03±0.15	NG[b]	2.47±0.31	0.23±0.40[c]
Verotoxigenic E. coli O157:H7	2.18±0.22	NG[b]	2.09±0.21	NG[b]	2.09±0.21	NG[b]
Listeria monocytogenes	2.27±0.56	NG[b]	1.57±0.56	NG[b]	1.09±0.19	NG[b]

[a]average of six tests ± standard deviation;
[b]no growth of viable colonies in TSA Petri dishes in all tests;
[c]no growth of viable colonies in TSA Petri dishes in one or two tests.

proteins and dissolving and breaking the polymeric compounds outside the bacterial membrane. This enhances the subsequent activity of the acidic components (AYEBAH *et al.*, 2005).

Test 3: EOW disinfectant activity after application on surfaces (pH, ORP and free chlorine)

The pH did not undergo marked changes over time, except for a small decrease over 6 h, whereas modifications in the ORP activity and free chlorine content of EOW occurred. As seen in Table 4, both parameters showed a fast reduction with time, reaching half of the initial value after 24 h in the case of ORP activity and almost a two-fold reduction in free chlorine content after a 6 h treatment.

Test 4: Influence of spraying on EOW chemical characteristics (pH and ORP)

Low-pressure spraying slightly influenced EOW characteristics and its physico-chemical properties (Table 4). The pH remained between 2.69 and 2.83 and ORP was reduced from 1,135 to 1,109 mV.

On the other hand, after application on two types of surfaces, the free chlorine concentration dropped rapidly over time, along with a parallel decrease of ORP which, in 24 h, reached the levels regularly present in tap water. The hypochlorous acid is the most active form of chlorine. It penetrates the bacterial cell and inhibits essential respiratory enzymes (FABRIZIO *et al.*, 2002);

it is 80 times more efficient than hypochlorite ion (KIM *et al.*, 2000). When hypochlorite is added to water, it generates undissociated hypochlorous acid. The hypochlorous acid dissociates into the anion hypochlorite in an alkaline environment and into chlorine gas in an acid environment (IZUMI, 1999). Thus, the chorine level in EOW tends to decrease when EOW is in contact with air due to the evaporation of chlorine gas or self-decomposition (LEN *et al.*, 2002).

CONCLUSIONS

The results of this study proved the efficacy of EOW in reducing the mesophilic bacterial contamination in operating conditions using short treatments (5 min) and using partially contaminated working equipment. The contamination levels in food industry environments are usually lower than that used in our study, especially when considering pathogenic bacteria. The efficacy of EOW to inactivate the entire population of pathogens present on surfaces might be even higher under natural working conditions. EOW can be considered a good alternative to hypochlorite, which must be used in large amounts and may be harmful to humans by intake via food (BACH *et al.*, 2006; FABRIZIO *et al.*, 2002; LIU *et al.*, 2006; SHARMA and DEMIRCI, 2003). EOW has been proven to be very unstable after application on surfaces which might make

Table 4 - Changes in EOW characteristics with time after spraying on steel and teflon surfaces.

Time	pH		ORP (mV)		Free chlorine (mg/L)	
	Steel	Teflon	Steel	Teflon	Steel	Teflon
0 min	2.69	2.69	1139	1139	110.76	110.76
1 h 30 min	2.81	2.67	1123	1150	31.8	33.7
3h 30 min	2.85	2.71	1127	1134	8.9	11.0
6 h	2.55	2.56	688	925	1.8	0.71
24 h	2.37	2.44	542	550	1.9	0.35

it unnecessary to rinse surfaces after its application and before the initiation of working activities, depending on the length of time between disinfection and onset of working activities. This would save time and, hence be more profitable.

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