ELECTROCHEMICALLY GENERATED SANITIZER FOR CLEAN-IN-PLACE

Challenge study of inoculated *Salmonella*, *Listeria*, *E. coli*, and *B. cereus* coupons spray treated with BIOIONIX sanitizer.

BIOIONIX

BIOIONIX ELECTROCHEMICAL SYSTEM

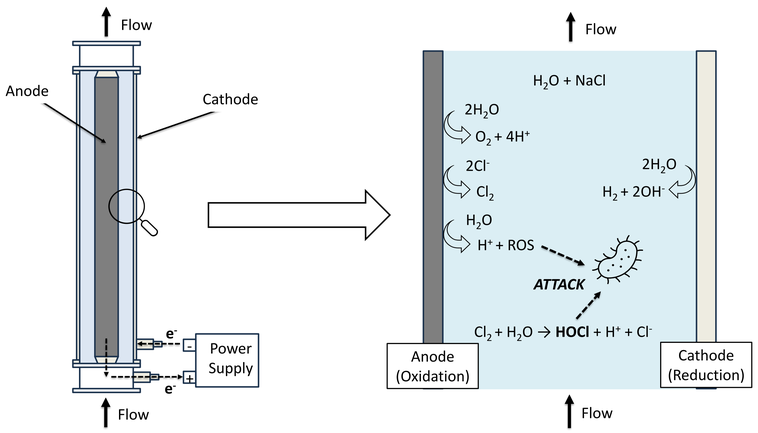
The BIOIONIX System

Electrolyte flows into the bottom of the system and passes through cylindrical anodes and cathodes. The electrolyte is a process water with a salt (NaCl) concentration of at least 0.25% and a pH between 5 and 7. Salt and pH adjustments are made by the system to keep these metrics within their operating ranges.

EPA Registered Device: 103663-WI-1

Some current process water applications:

- Fresh water with salt and acid addition
- Ocean water
- Cheese brines
- Ready-to-eat meats liquid smoke

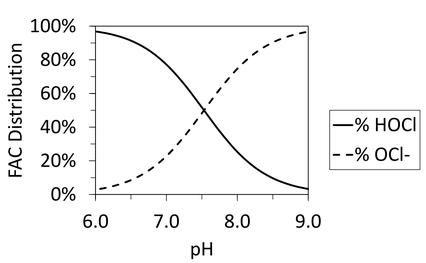


Chlorine Chemistry

Upon contact with water chlorine forms hypochlorous acid (HOCl) and hypochlorite (OCl-) depending on the pH of the solution. Free available chlorine (FAC) is the sum of OCl- and HOCl. HOCl is a strong oxidizer that, due to its small size and neutral charge, penetrates cell walls and destroys microbials.

$$[\text{FAC}] = [\text{OCl}^-] + [\text{HOCl}]$$

$$\text{OCl}^- + \text{H}^+ \rightleftharpoons \text{HOCl}$$



CHALLENGE STUDY PROCEDURE

Objective

Seeking a 3-log (99.9%) reduction in microbial counts of *Salmonella*, *Listeria*, *E. coli*, and *B. cereus* inoculated stainless steel and plastic coupons treated with BIOIONIX sanitizer after 30 sec spray.

Test Organisms

Inoculum was prepared by the plate harvest method with the cocktails created by combining equal dilution volumes into a single mixture.

- *Salmonella* spp.
- *Listeria monocytogenes*
- *Escherichia coli* O157:H7
- *Bacillus cereus* (spores were mixed with the vegetative cells for inoculation)

Test Surfaces

- Stainless steel: 4” x 4” 304 food-grade tiles
- Polystyrene: 100 x 15 mm medical grade, gamma sterilized petri dish

Inoculation

After the coupons were autoclaved, sets of inoculated coupons were prepared in triplicate for each of the organism and surface combinations.

- 1 mL of inoculated cocktail transferred and distributed evenly over 2” x 2” of the coupon’s surface.
- Inoculated coupons held uncovered at 30°C, 30% RH for 18 hrs. to dry and stabilize.

Sanitizer Application

- The BIOIONIX electrolyzed water sanitizing solution was prepared fresh and immediately sprayed on the inoculated coupons with a handheld sprayer.
- Coupons rested for 20 minutes before being transferred to sterile Whirl-Pak bags.
- Three inoculated coupons, per surface per organism were left untreated to determine the starting inoculation levels.

Electrolyzed Water Spray Characteristics	
Distance	15-20 cm
Time	30 sec
Flow	10 mL/s
Temperature	20°C
Pressure	65 psi
pH	5.5
FAC	150 ppm

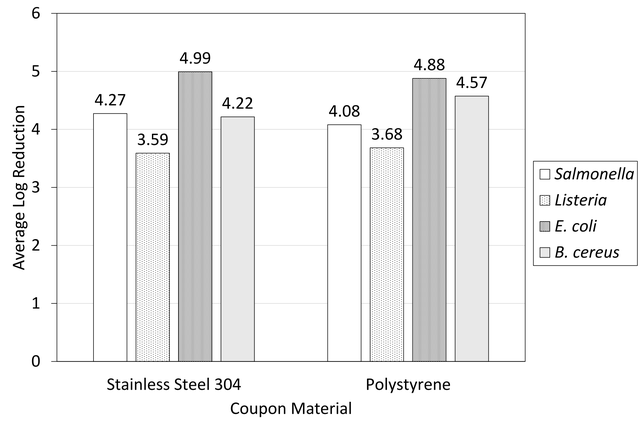
Dilution and Plating

Samples were analyzed aseptically by adding 10 mL of buffered peptone water and massaging the coupon surface for 30 sec. Further dilutions were performed in 0.1% peptone diluent and plated for the target organism.

Target Organism	Plating Media	Incubation
Salmonella	Tryptic soy agar (TSA) spread-plated with XLD overlay	35°C, 24 hrs.
Listeria monocytogenes	TSA spread-plated with Modified Oxford Medium (MOX) overlay	30°C, 48 hrs.
Escherichia coli O157:H7	Eosin Y Methylene Blue (EMB) overlay	35°C, 24 hrs.
Bacillus cereus	Spread-plated on to Bacara agar	30°C, 24 hrs.

RESULTS & DISCUSSION

The BIOIONIX electrolyzed water achieved average reductions exceeding the 3-log (99.9%) performance criterion for all pathogen–surface combinations. *Escherichia coli* O157:H7 exhibited the greatest susceptibility, while the more resistant *Listeria monocytogenes* consistently exceeded 3.5-log reduction on both surfaces.



The U.S. Food and Drug Administration recognizes sodium hypochlorite (NaOCl) as a broad-spectrum food-contact surface sanitizer at concentrations up to 200 ppm (21 CFR §178). NaOCl is commonly used in clean-in-place (CIP) systems to sanitize pipework following cleaning steps that typically involve caustic washes.

However, hypochlorite itself is not the primary antimicrobial species; rather, antimicrobial efficacy is driven by hypochlorous acid (HOCl) formed when chlorine is diluted in water (Fukuzaki, 2006). The BIOIONIX system operates at comparable FAC levels to sodium hypochlorite solutions but produces a substantially higher proportion of HOCl, resulting in enhanced antimicrobial activity.

In this challenge study, coupons were not subjected to a caustic cleaning step prior to sanitizer application, representing a conservative “worst-case scenario,” as surface-adhered and desiccated cells are more difficult to inactivate. Despite these conditions, significant microbial reductions were achieved within 30 seconds using a single spray application. Under typical CIP conditions, where pipework is flooded and sanitizer is recirculated, the BIOIONIX solution would be expected to serve as an effective and safe alternative to traditional chemical sanitizers.

REFERENCES

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