

Video Article

The Portable Chemical Sterilizer (PCS), D-FENS, and D-FEND ALL: Novel Chlorine Dioxide Decontamination Technologies for the Military

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Abstract

There is a stated Army need for a field-portable, non-steam sterilizer technology that can be used by Forward Surgical Teams, Dental Companies, Veterinary Service Support Detachments, Combat Support Hospitals, and Area Medical Laboratories to sterilize surgical instruments and to sterilize pathological specimens prior to disposal in operating rooms, emergency treatment areas, and intensive care units. The following ensemble of novel, 'clean and green' chlorine dioxide technologies are versatile and flexible to adapt to meet a number of critical military needs for decontamination^{6,15}. Specifically, the Portable Chemical Sterilizer (PCS) was invented to meet urgent battlefield needs and close critical capability gaps for energy-independence, lightweight portability, rapid mobility, and rugged durability in high intensity forward deployments³. As a revolutionary technological breakthrough in surgical sterilization technology, the PCS is a Modern Field Autoclave that relies on on-site, point-of-use, at-will generation of chlorine dioxide instead of steam. Two (2) PCS units sterilize 4 surgical trays in 1 hr, which is the equivalent throughput of one large steam autoclave (nicknamed "Bertha" in deployments because of its cumbersome size, bulky dimensions, and weight). However, the PCS operates using 100% less electricity (0 vs. 9 kW) and 98% less water (10 vs. 640 oz.), significantly reduces weight by 95% (20 vs. 450 lbs, a 4-man lift) and cube by 96% (2.1 vs. 60.2 ft³), and virtually eliminates the difficult challenges in forward deployments of repairs and maintaining reliable operation, lifting and transporting, and electrical power required for steam autoclaves.

Video Link

The video component of this article can be found at <http://www.jove.com/video/4354/>

Introduction

The PCS technology proceeds from where no commercial device existed previously and generates the disinfectant chlorine dioxide (ClO₂) that has a proven ability to kill vegetative pathogens on fresh produce^{3,6,9-13,15} or to decontaminate bacterial spores.^{6,14,15,17} The PCS has been laboratory validated specifically to effectuate sterilization against live cultures of *Geobacillus stearothermophilus* (GS) spores (see related reference⁹) and spore bio-indicators of *G. stearothermophilus* and *Bacillus atrophaeus* (BA)^{6,15,16}. The PCS has also been adapted to operate with less stringent conditions to ensure Food Safety by inactivating the vegetative pathogens *Listeria monocytogenes* and *Escherichia coli* on fresh produce, such as whole tomatoes, and to extend the shelf-life of fresh-cut produce, for example, by inactivating the polyphenoloxidase browning enzyme in sliced apples^{6,15}. To generate chlorine dioxide, the PCS uses novel effector chemistry that proceeds via oxidation-reduction reactions at near-neutral pH, thus eliminating the use of acids and the inherent difficulties of shipping, storing, handling, and disposing acidic wastes in far-forward military deployments^{1,2,4,17}. In addition to the military, the PCS can also be used by Homeland Security/Defense; during natural disasters (Superstorm Sandy, tsunamis, hurricane Katrina) that incapacitate access to power, potable water, and waste removal; on-site by emergency first-responders; and in community hospitals or schools during power outages (blackouts and brown-outs).

The Disinfectant-sprayer for Foods and Environmentally-friendly Sanitation (D-FENS) also uses effector chemistry (3 chemical components) and a 2-step mixing process (*i.* pre-concentration followed by *ii.* post-reaction dilution) to generate aqueous chlorine dioxide, primarily in a collapsible spray bottle for decontaminating surfaces of Army materiel, food handling equipment and field feeding equipment in Army field kitchens and sanitation centers and Navy Galleys, medical units, showers, and latrines anywhere large numbers of deployed personnel co-exist in close proximity^{5,6}. Validation testing showed that D-FENS eliminates the pathogen *Staphylococcus aureus*, a common foodborne pathogen, on porous surfaces¹⁴. "D-FEND ALL" (Disinfectant for Environmentally-friendly Decontamination, All-purpose) provides a simpler (2 chemical components), more convenient (1-step mixing) alternative with unmatched versatility for producing aqueous chlorine dioxide to decontaminate bacterial spores on textiles, for surface disinfection to promote sanitation and hygiene, and to improve water quality and safety, with particular advantages for

applications requiring the rapid production of large volumes of dilute chlorine dioxide solutions using small quantities of starting materials for applications in novel graywater recycling technologies designed to generate clean, potable water for Expeditionary Base Camps².

A variety of mechanisms exist in accordance with the Federal Technology Transfer Act to facilitate the transfer of federal technologies to nonfederal entities as a way to encourage the development and commercialization of technologies for the material benefit of the nation. Accordingly, with their burgeoning potential for many military and civilian uses, the PCS, D-FENS, and D-FEND ALL technologies have been patented and transferred to industry for commercialization via Patent Licensing Agreements and Commercial Evaluation Licenses. A slow, controlled release version of D-FENS (called "D-FENS Lite") was Technology Transferred to commercial industry for incorporation into packaging materials to extend the shelf-life of fresh berries, and the PCS has also been Technology Transferred to academia and other government agencies for comparative testing with other technologies, for research on Food Safety with fresh produce commodities, and for enhancing undergraduate science education. The Technology Transfer of the PCS and its chemistry led to a commercial product approved for bio-hood sterilization with improvements in time, cost, and environmental protection compared to conventional formaldehyde treatments.

Protocol

1. The Portable Chemical Sterilizer (PCS)

1. Equipment. The PCS is an innovative device for portable, energy-independent, point-of-use medical sterilization. For these purposes, a commercial PELICAN rigid plastic suitcase was embellished with special design features to accommodate sterilization (**Figure 1**).
2. Equipment Design. a) A wide-mouthed reaction vessel receives the dry chemical reagents and water; b) two check valves installed in the case wall relieves pressure at 1 psi; c) a filtered inlet valve allows air to be pumped in for flushing the chamber; d) a circulating tube distributes air through the chamber post-sterilization; e) disposable dry scrubber (activated carbon) devices engrafted over the outlet check valves remove residuals and ensure the health and safety of the user and environment; and f) stilts in the base of the case accommodate a surgical instrument tray or other perforated tray and maximize gas-flow during flushing.
3. Operation. Place the PCS on a level surface and open the lid. Place a surgical tray containing clean, non-sterile instruments and wrapped in blue autoclave paper on the stilts inside the case. Mix dry chemicals and water (e.g., 93 g sodium chlorite, 63 g sodium sulfite, 25 g sodium hydrogen ascorbate, and 300 ml water – other permutations are possible) in the wide-mouthed reaction vessel to initiate chemical reaction, then close and lock the lid. In under 2 min, the reaction produces copious chlorine dioxide sterilant, heat, and humidity. At 25 min, connect a battery-operated or hand-powered air pump to the inlet valve and flow air into the chamber for approx. 5 min (**Figure 2**).
4. Cycle Completion and Re-use. Open the case and remove the surgical tray of sterile instruments. Dispose of the reaction vessel (water and benign chemical salts). The PCS is available for immediate re-use with another tray of surgical instruments and a fresh set of dry chemicals and water.
5. Validating Sterilization with Bio-indicators. Place commercially available B/T Sure Biological Indicators containing spores impregnated on paper ($\sim 10^5$ spores/unit) of either *G. stearothermophilus* (used for wet heat) or *B. atrophaeus* (used for ethylene oxide gas sterilization) inside the case for a sterilization cycle. At completion of the ClO₂ sterilization cycle, remove and activate the indicators, then incubate indicators for 24-48 hr to validate sterilization.
6. Validating Sterilization with Spore Suspensions. Place aqueous suspensions of *G. stearothermophilus* spores ($\sim 10^5$ cfu/ml) inside the PCS for exposure to a ClO₂ sterilization cycle. Recover *G. stearothermophilus* spores exposed to the chlorine dioxide treatments on Antibiotic Assay Medium with 1% soluble starch⁸ (no recovery indicates sterility). Examine refractility of treated spores with phase contrast microscopy (spores inactivated by ClO₂ retain phase bright character¹⁴).
7. Validating Sterilization of Hard Surfaces. Inoculate hard, non-porous surfaces made of glass or metal with aqueous suspensions ($\sim 10^5$) of *G. stearothermophilus* spores. Place inoculated materials into the PCS for treatment with a ClO₂ sterilization cycle. Sampling the treated surfaces with commercially available Difco HY-Check swabs and obtaining no-growth confirms sterility.

2. "D-FENS"

1. Equipment. "D-FENS" is a collapsible handheld bottle fitted with a hand-operated spray-trigger device. The flexible plastic bottle has a gusseted bottom to stand-up when full, and the chemically-resistant plastic affords multiple re-uses per sprayer (**Figure 3**).
2. Generate Aqueous ClO₂ solution. D-FENS uses 1-10 g total quantity of dry reagents to generate up to 800 ml of 50 – 500 ppm chlorine dioxide solution (e.g., 4.7 g sodium chlorite, 1.6 g sodium sulfite, and 1.3 g sodium ascorbate, with permutations possible). Use "kinetics control," a novel 2-step mixing process comprising: *i. Pre-concentration* – dissolving all reagents in a small volume, and *ii. Post-reaction dilution* – diluting the solution to its final working volume, to generate aqueous chlorine dioxide solutions in the spray-bottle in 2-9 min. The disinfectant solution is sprayed as a fine mist or aerosol to disinfect or decontaminate intended surfaces. The chlorine dioxide solution in D-FENS remains stable for a minimum 8 hr shift and any remaining solution can be emptied down sink or floor drains at the end of a shift to purge biofilms.
3. Microbiological Validation – inoculating porous surfaces. Prepare Petri dishes of Baird-Parker Agar (BPA) containing egg yolk tellurite (EYT) and Yeast Extract (YE) and inoculate agar surface with 0.1 ml of a $\sim 10^6$ cfu/ml suspension of a 3-strain cocktail of *Staphylococcus aureus* (*S. aureus* A-100 that produces enterotoxin A, *S. aureus* ATCC 14458 that produces enterotoxin B, and *S. aureus* 993 that produces enterotoxin D)⁷. *S. aureus* was selected as the target organism because it produces distinctively obvious black colonies, if not inactivated.
4. Microbiological Validation – Testing Porous Surfaces. Using the D-FENS spray bottle containing chlorine dioxide solution, spray the disinfectant solution onto the porous agar surface. Use consistent, steady force to dispense approximately equal volumes of solution per spray-trigger pulse. Rotate the plates 90° between successive pulses to apply uniform coverage of the agar surface. Also use this technique with a glass hockey stick and applying light pressure to spread chlorine dioxide solution over the agar surface for mechanical abrasion (equivalent to wiping or scrubbing – see **Figure 4**).
5. Microbiological Validation – Hard Surfaces. Inoculate sterilized custom stainless steel (type 304) coupons (4" x 4", with total surface area 10.16 cm²) with 0.2 ml volume of aqueous suspensions of bacterial cells (e.g., *S. aureus*, *Escherichia coli*, or *Listeria monocytogenes*) and spread inocula uniformly across coupon surface. Air-dry coupons for 30 min at room temperature in laminar flow hood. Pick up inoculated coupons with sterile forceps and immerse in 20 ml test solution containing chlorine dioxide in a 100 ml stomacher bag. After contact times of

0.5 to 5 min, quench the sanitizer solution by adding a small amount of sodium sulfite solid, then masticate solution for 2 min in a stomacher. Remove bag and in a laminar flow hood withdraw supernatant and serially dilute solution onto pre-made agar plates, then incubate for 24 hr to enumerate survivors.

3. The PCS for Fresh Fruits and Vegetables

The ability of reduced PCS treatments to kill harmful foodborne pathogens (*E. coli* and *L. monocytogenes*) on fresh produce was tested using a spot-inoculation method in which high levels of pathogens were spotted onto the exterior surfaces of tomato wedges.

1. Inoculation. Inoculate the exterior surfaces of 25 gram samples of tomato wedges with either 10^5 CFU/g *L. monocytogenes* OSY-8578 or with 10^6 CFU/g *E. coli* ATCC 11229, then air-dry in a sterile bio-hood for 15 min.
2. PCS treatment. After the inoculum dries, place the tomato wedges (wear sterile gloves) in the PCS and test under various conditions of chlorine dioxide concentration and exposure time. In some instances, place spore bio-indicators of *G. stearothermophilus* and *B. atrophaeus* inside the PCS to accompany the tomato wedges and validate the sterilization treatment (**Figure 5**).
3. Microbial Recovery. After the PCS treatment, place tomato wedges in a stomacher bag with 50 ml of aqueous phosphate buffer (pH 7), then masticate for 2 min with a stomacher blender.
4. Enumeration. Dispense the masticated solution as serially 10-fold dilutions onto agar plates Tryptic Soy Agar-Yeast Extract (TSAYE) and nutrient agar (NA) for *L. monocytogenes* and *E. coli*, respectively, and spread with a glass hockey stick, cover and incubate plates at T = 35 °C for 24-48 hr. Enumerate survivors using a colony counter to confirm microbial inactivation.
5. Inactivating Polyphenoloxidase (“browning”) Enzymes. Place uninoculated apple slices in separate Petri dishes inside the PCS and expose to chlorine dioxide (**Figure 5**). After treatment, remove the Petri dishes and expose the apple slices in the ambient environment. Visual observation showed no browning for up to 1 week post-treatment.

4. “D-FEND ALL”

1. Generate Aqueous ClO₂ solution. D-FEND ALL uses small quantities of dry chemicals (chlorite and SAMIA) in water to generate chlorine dioxide solution in 0.5-3.0 min. For example, mix 0.8-3.3 g of reagents in 15-1,200 ml of aqueous solution to produce a chlorine dioxide solution.
2. Microbiological Validation – Textiles. Inoculate sterile strips of textile samples with aqueous suspensions of *Bacillus anthracis* Sterne or *Bacillus amyloliquefaciens* spores, and let textile strips air-dry in a laminar flow hood. Pick up strips with sterile forceps and immerse in 20 ml of chlorine dioxide solution in a 100 ml stomacher bag. At 10 min, quench process without affecting spores by adding a small amount of sodium sulfite solid, then masticate textile strip and solution for 2 min in a stomacher. Remove bag and in a laminar flow hood serially dilute solution onto pre-made agar plates, then incubate for 24 hr and enumerate to validate decontamination.

Representative Results

The easy-to-operate PCS was designed to achieve sterility by inactivating bacterial spore suspensions or bacterial spore bio-indicators in 30-minute treatments involving the controlled production of chlorine dioxide by unique effector chemistry. Specifically, microbiological validation studies verified that the PCS achieved sterility by inactivating bio-indicators containing spores (10^5 spores/ml) of either *G. stearothermophilus* or *B. atrophaeus*, that are intended to indicate sterilization by steam autoclaves (wet heat) or ethylene oxide gas, respectively. The PCS also sterilized suspensions of *G. stearothermophilus* spores and *B. atrophaeus* spores configured as suspensions or dried on hard, non-porous surfaces made of glass or metal and recovered using commercial Difco HY-Check swab kits. *G. stearothermophilus* spores exposed to the chlorine dioxide treatments were not recoverable but retained their phase bright character after treatment, indicating that the mechanism of spore inactivation by chlorine dioxide is different from wet heat or high pressure and most likely involves damage to the spore's inner membrane¹⁷.

The PCS was adapted for less stringent conditions of ClO₂ generation to treat fresh produce to achieve food safety without compromising food quality. Specifically, spot-inoculating high levels of the pathogens *E. coli* ATCC 11229 (10^6 CFU/g) and *L. monocytogenes* OSY-8578 (10^5 CFU/g) on the exterior surfaces of 25 g samples of tomato wedges, air-drying the samples in a sterile bio-hood for 15 min, placing them inside the PCS, and testing them under various conditions of ClO₂ concentration and exposure time (**Figure 5**) confirmed the efficacy of the PCS for fresh and fresh-cut produce. *G. stearothermophilus* and *B. atrophaeus* spore bio-indicators and uninoculated apple slices were placed in the PCS during treatment. Treatment conditions were found that inactivated the target microorganisms on whole tomato surfaces to render safe-to-eat tomatoes free from these foodborne pathogens without compromising the red color of the tomatoes (**Table 1**). Sliced apples treated in the PCS did not exhibit browning of the pulp tissue when exposed to ambient air subsequently, but remained white for at least a week. The PCS treatment inactivated the polyphenoloxidase enzyme that causes cut apple tissue to turn brown. Also, the apple skin tended not to discolor by exposure to the ClO₂.

In addition to inactivating enzymes and vegetative pathogens, ClO₂ also inactivates bacterial spores such as *Bacillus anthracis* (causative agent of ‘Anthrax’) for bio-weapon decontamination. **Figure 6A** shows the use of Atomic Force Microscopy (AFM) to characterize ClO₂-treated *B. subtilis* spores. The ClO₂-treated spores remained intact and did not collapse upon air-drying. These high resolution AFM images also show that the spore coat architecture and topology were unaltered by the ClO₂ treatment. For purposes of illustration, **Figure 6B** shows an electron micrograph of a dormant *Bacillus cereus* spore with a prominent exosporium layer, which is also characteristic of *B. anthracis* spores.

Validation experiments showed that the D-FENS sprayer system completely inactivated the infectious bacteria *S. aureus*, as evidenced by the prevention of growth of black colonies on BPA. In similar tests, 100 ppm chlorine dioxide solutions effected a > 7-log reduction of a 3-strain cocktail of *S. aureus* inoculated onto stainless steel surfaces after 1, 3, and 5 min of contact. D-FEND ALL provides additional versatility as a convenient method for generating ClO₂ with specific advantages for using it in dilute concentrations for applications such as water safety,

wastewater disinfection, and textile decontamination. Validation experiments with D-FEND ALL showed the inactivation of bacterial spores without damaging the textile material.

In comparative testing of D-FENS with three commercially available generators which rely on electrochemical cells and selectively permeable membranes to produce different chemical sanitizers (Electrolyzer, WaterStar and Tersano). In these cases, soft water is recommended for use due to the potential for mineral deposits (slaking) that can damage electrochemical cells. **Table 2** provides details about each system's active agent and the advantages of D-FENS in terms of efficacy, power consumption, weight, size, and reduced capital costs compared to other technologies. A concentration of 100 ppm ClO_2 was chosen for the D-FENS system (D-FENS can be adjusted to produce ClO_2 concentration in the range of 5-4,000 ppm). The D-FENS solution effected a > 7-log reduction of the 3-strain cocktail of *S. aureus* inoculated onto stainless steel surfaces in contact times as short as 1 min (**Table 3**). These results demonstrate that D-FENS is an effective disinfectant spray to reduce *S. aureus* from hard, non-porous contact surfaces in the food preparation and handling environment, such as counter tops, cutting boards, utensils, etc.



Figure 1. The PCS prototype derives from a rigid plastic PELICAN™ suitcase embellished with several design features to ensure sterilization while controlling pressure and temperature, and without off-gassing into the environment¹⁵.



1. Mix reagents



2. Generate ClO_2



3. Flush

Figure 2. The PCS operates via an easy 3-step procedure. Step 2.1: add dry chemical reagents and water in vessel and close suitcase, Step 2.2: let reaction generate ClO_2 and treat for 25-60 min, and Step 2.3: air flush through scrubber, then retrieve sterile surgical instruments¹⁵.



Figure 3. The D-FENS sprayer (left) generates aqueous ClO_2 on-site and at point-of-use in a spray-bottle and easily sprays ClO_2 onto contact surfaces (right) to wipe away contaminating pathogens in Army Field Kitchens (center top and bottom)¹⁵.



Figure 4. Visible growth of *S. aureus* colonies on untreated agar surfaces (left), and “no-growth” on agar surfaces treated with the D-FENS ClO_2 sprayer.



Figure 5. A typical PCS set-up to inactivate *E. coli* or *L. monocytogenes* on exterior tomato surfaces (center), browning enzymes in apple slices (right), and spore bio-indicators (back left and back right)¹⁵.

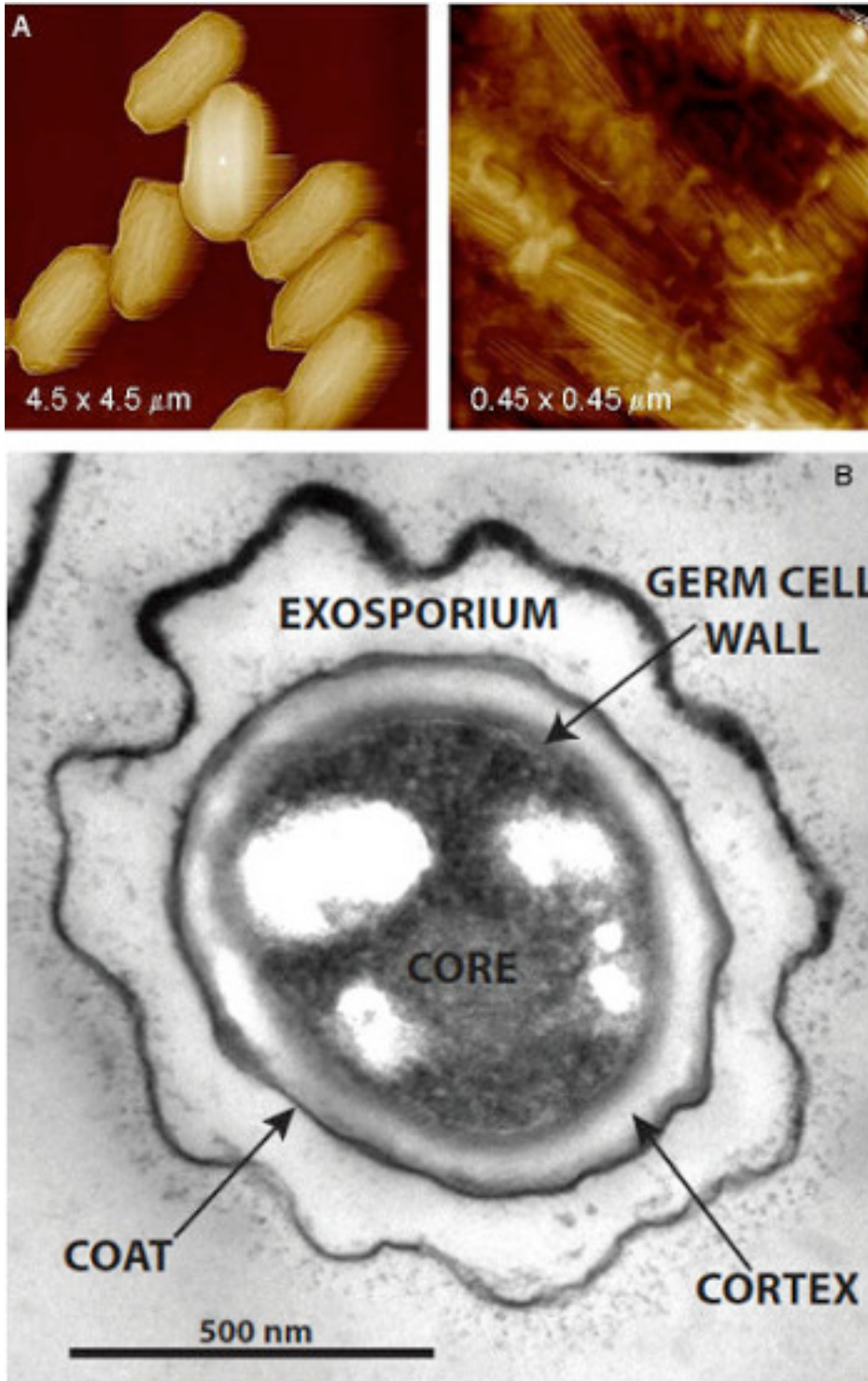


Figure 6. A) AFM of ClO₂-treated *B. subtilis* spores. B) Transmission EM of *B. cereus* spores with a prominent exosporium¹⁴.

ClO ₂ treatment	Time (min)	GS indicator	BA indicator	<i>Listeria</i> ^a (10 ⁵)	<i>E.coli</i> ^b (10 ⁶)
I	30	neg. (×3)	neg. (×2)		
II	30	neg. (×1)	neg. (×1)		neg. (×3)
III	60	neg. (×1)	neg. (×2)	neg. (×2)	

Table 1. PCS treatments with different ClO₂ concentrations (I > II > III) of *G. stearotherophilus* (GS) and *B. atrophaeus* (BA) spores and vegetative pathogens (negatives indicate no survivors post-treatment)¹⁵.

- a. 25g tomato wedges inoculated with *Listeria monocytogenes* OSY- 8578
- b. 25g tomato wedges inoculated with *Escherichia coli* ATCC 11229

Technology	Active agent	Power (W)	Weight (lbs)	Dimensions
ElectroCide (Electrolyzer Corp.)	HOCl	84	40	18" × 12.5" × 6.5
FC-2 (WaterStar)	H ₂ O ₂	120	6.7	12" × 9" × 15"
Lotus (Tersano)	O ₃	60	21	12" × 7" × 12"
SAL-40 (MIOX)	Mixed oxidants	84	150	67.3" × 29" × 9.5"
"D-FENS"	ClO ₂	0	< 1	1 Liter pouch

Table 2. Sanitizing Solution Generators.

	Before Treatment	After Treatment			
Disinfectant	ppm	pH	CFU before	CFU after	log reduction
		<i>S. aureus</i>			
HOCl	88.0	3.0	3.4 × 10 ⁷	0.0	7.5
Clorox	200.0	10.1	3.4 × 10 ⁷	0.0	7.5
H ₂ O ₂			3.4 × 10 ⁷	3.2 × 10 ³	3.4
O ₃			1.8 × 10 ⁸	3.2 × 10 ³	4.8
ClO ₂	100	6.7		0.0	> 7

Table 3. Efficacy of sanitizers on cells adhered to stainless steel surface.

* This value holds for contact times of 1, 3, and 5 min. Data provided courtesy of Edmund Powers, US Army – NSRDEC.

Discussion

This foundational R&D has set new research and technical directions through collaborations with academia, other Government agencies, and industry that have led to the commercialization of novel, environmentally-friendly ("green") technologies. Chlorine dioxide is the first method approved by the National Sanitation Foundation in 20 years for safer, faster, and more environmentally-friendly sterilization than conventional treatments. The PCS, D-FENS, and D-FEND ALL prototypes have been validated as bench-scale prototypes in a laboratory environment, and the range of results imparts these systems with tremendous breadth of versatility to flexibly accommodate the needs of individual users. At this stage, prototypes have been patented and licensed to commercial industry for production and commercialization – commercial products based on these technologies are currently available in the marketplace. Industry partners are needed to make these technologies a reality for the military (and for Homeland Defense/Security, Global Disaster Relief, and Third-World Aid – "Doctor's Without Borders"), first by determining the application, configuration, and packaging as a salable mixed-chemical technology. For the PCS, FDA clearance testing will be required as a medical sterilization device, but should be attainable, and the resultant device would be procurable by military agencies and have benefits for medical/surgical devices made of plastic, with bendable optics, or with small lumens that are otherwise susceptible to damage by conventional wet heat treatments of "Bertha" autoclaves. D-FENS and D-FEND ALL are convenient spray-and-wipe applications of aqueous chlorine dioxide that eradicate from surfaces vegetative pathogens, viruses, resistant bacterial spores, and bio-films on surfaces to meet important Army needs for decontamination. This corpus of research and development encompasses 15 patents/patent applications, more than seven (7) Technology Transfer agreements, and the 2009 IFT Press/Wiley-Blackwell book "Microbial Safety of Fresh Produce."

Together, the PCS, D-FENS, and D-FEND ALL provide a versatile and adaptable arsenal of ClO₂ technologies for eliminating vegetative pathogens and bacterial spores in myriad applications for surgical instrument sterilization, textile decontamination, fresh and fresh-cut produce sanitization, and hard surface decontamination anywhere microbial contamination is an issue. Thus, invented and validated at the US Army Natick Soldier RDEC laboratories to **empower** Soldiers with germ-killing strength, to **unburden** logistics by eliminating the need to transport water, and to **protect** Soldiers and the environment by decreasing fossil fuel consumption during shipping, reducing CO₂ emissions and carbon footprint, and decreasing landfill wastes, these technologies have been commercialized and are available for dual-use applications for civilian consumers.

Disclosures

We have no further disclosures.

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References

1. Curtin, M. A., Taub, I. A., Kustin, K., Sao, N., Duvall, J. R., Davies, K., Doona, C. J., Ross, E. W. Ascorbate-induced oxidation of formate by peroxodisulfate: product yields, kinetics and mechanism. *Research on Chemical Intermediates*. **30** (6), 647-661(2004).
2. Curtin, M. A., Dwyer, S., Bukvic, D., Doona, C. J., Kustin, K. Kinetics and mechanism of the reduction of sodium chlorite by sodium hydrogen ascorbate in aqueous solution at near-neutral pH. *International Journal of Chemical Kinetics*. **46**(4), 216-219(2014).
3. Doona, C.J., Curtin, M. A., Feeherry, F. E., Kandlikar, S., Baer, D., Kustin, K., Taub, I. A., McManus, A. T. *Portable Chemical Sterilizer.*, U.S. Patent Number 7,625,533 (2009).
4. Doona, C.J., Curtin, M. A., Taub, I. A., Kustin, K. *Chemical Combination for the Generation of Disinfectant and Heat.*, U.S. Patent Number 7,883,640 (2011).
5. Doona, C.J., Feeherry, F. E., Kustin, K., Curtin, M. A. *Process for producing aqueous chlorine dioxide for surface disinfection and decontamination.*, U.S. Patent Application Number 8,337,717 (2012).
6. Doona, C.J., Feeherry, F. E., Kustin, K., Feng, H., Grove, S., Krishnamurthy, K., Lee, A. Combining sanitizers and nonthermal processing technologies to improve fresh-cut produce safety. In: *Electron beam pasteurization and complementary food processing technologies*. (S. D. Pillai and S. Shayanfar, S., Eds.), Woodhead Publishing, Cambridge (2014).
7. Feeherry, F. E., Doona, C. J., Taub, I. A. Effect of water activity on the growth kinetics of *Staphylococcus aureus* in ground bread crumb. *Journal of Food Science*. **68** (3), 982(2003).
8. Feeherry, F.E., Munsey, D. T., Rowley, D. B. Thermal inactivation and injury of *Bacillus stearothermophilus* spores. *Applied and Environmental Microbiology*. **53** (2), 365 (1987).
9. Gómez-López, V.M., Devlieghere, F., Ragaert, P., Debevere, J. Shelf-life extension of minimally processed carrots by gaseous chlorine dioxide. *International Journal of Food Microbiology*. 116, 221 (2007).
10. Mahmoud, B.S.M., Bhagat, A. R., Linton, R. H. Inactivation kinetics of inoculated *Escherichia coli* O157:H7, *Listeria monocytogenes*, and *Salmonella enterica* on strawberries by chlorine dioxide gas. *Food Microbiology*. **24** (7-8), 736 (2007).
11. Mahmoud, B.S.M., Linton, R. H. Inactivation kinetics of inoculated *Escherichia coli* O175:H7 and *Salmonella enterica* on lettuce by chlorine dioxide gas. *Food Microbiology*. **25** (2), 244 (2008).
12. Kim, Y.-J., Lee, S.-H., Park, Ji., Park, Jo., Chung, M., Kwon, K., Chung, K., Won, M., Song, K. B. Inactivation of *Escherichia coli* O157:H7, *Salmonella typhimurium*, and *Listeria monocytogenes* on stored iceberg lettuce by aqueous chlorine dioxide treatment. *Journal of Food Science*. **73** (9), M418-M422 (2008).
13. Park, E.-J., Gray, P. M., Oh, S.-W., Kronenberg, J., Kang, D.-H. Efficacy of FIT produce wash and chlorine dioxide on pathogen control in fresh potatoes. *Journal of Food Science*. **73** (6),M278-M282. (2008).
14. Setlow, P. Bacterial Spores. In: *Industrial Pharmaceutical Microbiology*. (N. Hodges and G. Hanlon, Eds). Euromed Communications, Supplement 10, England (2011).
15. Setlow, P., Doona, C. J., Feeherry, F. E., Kustin, K., Sisson, D., Chandra, S. Enhanced Safety and Extended Shelf Life of Fresh Produce for the Military. In: *Microbial Safety of Fresh Produce*. (X. Fan, B.A. Niemira, C.J. Doona, F.E. Feeherry, and R. B. Gravani, Eds.). IFT Press Wiley Blackwell, Ames, IA, pp. 263-288 (2009).
16. Taub, I.A., Roberts, W., LaGambina; S. Kustin, K. Mechanism of Dihydrogen Formation in the Magnesium–Water Reaction. *Journal of Physical Chemistry*. **106** (35), 8070 (2002).
17. Young, S.B. and Setlow, P. Mechanisms of killing of *Bacillus subtilis* spores by hypochlorite and chlorine dioxide. *Journal of Applied Microbiology*. **95** (1), 54 (2003).