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## Development and Validation of a Pilot Scale Enhanced Biosafety Level Two Containment for Performance Evaluation of Produce Disinfection Technologies

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### Abstract

The development and validation of the Biosafety Level 2 (BSL-2) enhanced containment system located at the produce pilot plant facility of the U.S. Department of Agriculture—Eastern Regional Research Center is presented here. This multi-purpose containment is used to enclose pilot and pilot scale washing and sanitizing equipment for fruits and vegetables, or other decontamination equipment where aerosol generation is likely, and complete protection is required for researchers. This containment is operated under a negative pressure with all exhausted air (approximately eight containment air exchanges per hour) being passed in parallel through two hydrophobic HEPA filters. During operation, personnel are

excluded from the containment, materials are introduced into and removed from the containment via pass-throughs, and equipment is operated via computer control and glove ports.

At the completion of any series of processing trials, vegetative bacterial cells remaining within the containment, and in the processing water, are inactivated by raising the internal temperature of the containment, and all contents, including equipment, processing water and waste water to sufficiently inactivate all bacterial cells, except spore formers, using industrial steam at atmospheric pressure. We have demonstrated the feasibility and safety of conducting multiple trials using pathogenic bacteria with pilot scale processing equipment within the containment, and then inactivating surviving vegetative bacterial cells with the steam-in-place proc-

ess. Furthermore, we validated pilot-scale surface pasteurization processing equipment capable of significantly reducing cell densities (in excess of 5 logs) of *Salmonella* on inoculated cantaloupes, using this BSL-2 enhanced containment system.

## Introduction

The demand by consumers for fresh and fresh-cut fruits and vegetables has steadily increased due to the nutritious qualities associated with fresh produce, and the convenience of ready-to-eat fresh foods. This increased demand has resulted in increased per capita consumption of fresh fruits and vegetables in the U.S. (ERS, 2004). The market for packaged salads and fresh-cut fruits and vegetables is estimated at \$10 to \$12 billion annually (IFPA, 2004). Unfortunately, as produce consumption has increased in the U.S., so has the number of produce-related outbreaks of foodborne illnesses. Produce-related outbreaks accounted for 12% of all reported foodborne outbreaks in the 1990s compared to only 0.7% in the 1970s (USFDA, 2004; Sivapalasingam et al., 2004). The Centers for Disease Control and Prevention (CDC) reported that foodborne outbreaks associated with fresh produce doubled between the periods 1973 to 1987 and 1988 to 1992 (Buck et al., 2003). The continuing occurrence of such produce-related outbreaks may jeopardize further increases in per capita consumption due to lowered confidence in the microbial safety of the product by the consuming public. Such outbreaks can also have a significant economic impact on growers, processors, shippers and food service companies. The current cost in medical care and decreased productivity due to foodborne illnesses has been estimated to be between \$10 to \$83 billion each year (USFDA, 2004).

Federal initiatives to improve the microbiological safety of fresh produce generally call for improvements in post-harvest washing and sanitizing procedures. Current technologies available for use by the produce industry result in no more than 2 to 3 log (99-99.9%) reductions in pathogen levels, and fall short of meeting the U.S. Food and Drug Administration's (USFDA) target of 5 log (99.999%) reductions. Thus, more effective decontamination technologies and decontamination equipment are needed. Those interventions found to be effective in the laboratory should be scaled up in a pilot plant facility to facilitate the commercialization of the technology. However, human pathogens could not be introduced into a pilot plant, in its original configuration, for testing of pilot and commercial scale equipment, because of concerns for the safety of equipment operators, or other personnel, in the pilot plant area, and researchers found it necessary to use non-pathogenic surrogate organisms in place of the target pathogenic organism (Eblen et al., 2005). Such studies were inherently limited because surrogate organisms do not necessarily behave in a manner identical to that of their pathogenic counterparts. A pilot

plant, designed to accommodate pilot scale experiments using human pathogens, was required to facilitate validation and commercialization of new technologies for decontaminating produce containing human pathogens.

To create such a facility, the Eastern Regional Research Center (ERRC), Food Safety Intervention Technologies Research Unit (FSIT) and the Pennsylvania State University (PSU) entered into a Cooperative Agreement to design and fabricate a novel Biosafety Level 2 (BSL-2) enhanced containment. This primary barrier would enclose pilot-scale, or commercial produce processing equipment, and could be operated and decontaminated without the need for special personnel protection clothing, or equipment. The selection of decontamination process was based on the following criteria: the process should be environmentally friendly; should penetrate into the core of motors and other inaccessible locations; should produce no antimicrobial chemical residues that might confound subsequent processing trials.

Pressurized steam was selected for decontaminating the BSL-2 enhanced chamber. The BSL-2 enhanced chamber was required to be capable of confining human pathogens on inoculated produce undergoing different decontamination wash treatments utilizing various pilot and commercial scale processing equipment, to facilitate research on new washing and sanitizing treatments that can benefit the fresh produce industry, while providing protection to the researchers conducting such studies. This manuscript describes the design, fabrication, and validation of a unique BSL-2 enhanced containment, pilot-scale produce washing equipment, and associated remote controls and decontamination systems.

## Materials and Methods

### Containment Design

The containment structure is a modular design to facilitate construction, transportation, and expansion by adding additional modules end-to-end. Currently, the system consists of two modules (Figures 1 and 2). Each module (approximately cubical) was constructed with a welded frame of square 304 stainless steel tubing. The tubes within each module, and each door were joined by welding so that the inside of all the tubes in each door, or module forms a single continuous enclosed cavity. These four frame cavities (two modules and two doors) are filled with pressurized steam (207 kPa) during the decontamination process, with the steam entering through a pipe at the top of the containment and the condensate leaving through a steam trap diagonally across the containment. This pressurized steam-filled frame is considered the key feature of the design, and it ensures that the corners, which would otherwise be difficult to thermally decontaminate, are fully heated. In fact, because pressurized steam is used, the temperatures of the corners become hotter than the inside of the containment where only

atmospheric pressure steam is used. The bottom, top, and doors of the containment are all enclosed with panels welded to the frame. The panels are folded at approximately 70° to minimize the stress between the frame and the panel due to differential expansion when the frame is filled with steam. The panels in the bottom are sloped so that the water drains to an edge point fitted with a drain pipe, which leads to the drain decontamination system. On the top of each module, the center area was fitted with a removable stainless steel panel clamped to the frame. The containment frame and metal panels were covered with sheets of aluminum-clad insulation on most outside surfaces to protect personnel from hot surfaces capable of causing burns, and to conserve energy.

The containment sides are constructed of clear Lexan polycarbonate sheets (Part #43029, U.S. Plastics, Lima, Ohio, USA), and are held in place with linear clamping mechanisms that permit thermal growth without inducing stress on the panels. These polycarbonate panels provide high visibility of the inside of the containment chamber during operation. Hollow silicone bulb seals, (Part #1129A3, McMaster-Carr, Dayton, New Jersey, USA) were attached to the ends of the modules to provide a seal between modules, and between the module and each door (Figure 2). Like the frame, the gaskets are also filled with pressurized steam (35 kPa) while the containment is

in operation. This serves to prevent the possibility of leaking contaminated water that might accumulate on the gasket and would decontaminate the accumulated water due to the high temperature of the gasket.

Large produce processing equipment to be placed in the containment is normally outfitted with lockable casters. The equipment is rolled in through either of the large doors on the ends of the containment (Figure 1). Inside, the casters are supported and guided by two parallel channels (Figure 2). A movable perforated-metal grid walking platform is laid parallel to, and on either side of the equipment, to enhance personnel access to the equipment prior to closing the containment. Electric power and control circuits to the equipment are provided by weather-tight electrical cords and fittings, which enter the containment through weather-tight cord grips located in the removable ceiling panels of the containment.

The containment was designed to accommodate processing equipment that generates considerable heat and humidity. Therefore, a fan coil unit heat exchanger (Model #CB-12-CDW, Concepts and Designs Inc., Owatonna, Minnesota, USA) was installed to condense the water vapor and remove the heat (Figure 2). The all-stainless steel unit is suspended from the ceiling of one of the modules and is supplied with circulating chilled methanol solution available in the building. The conden-

**Figure 1**

BSL-2 Containment: A - Liquid Pass-Through (LPT); B - Aluminum Clad Insulation (typical); C - Computer Control Cabinet; D - Dry Pass-Through (DPT); E - HEPA Filters; F - Equipment Loading Doors



**Figure 2**

Axial View of BSL-2 Containment: A - Dry Pass-Through (DPT); B - Liquid Pass-Through (LPT); C - Fan Cooling Unit; D - Process Equipment; E - Silicone Gasket; F - Perforated Metal Grid Walking Platform; G - Equipment Support Channels; H - Drain Pan



sate drains from the unit discharge onto the floor of the containment. The reduction of humidity is particularly useful in minimizing obstruction of vision due to condensation on the windows.

During produce decontamination studies using the BSL-2 enhanced containment, produce samples can be moved in and out of the containment using dry and liquid pass-throughs, respectively, which are mounted in the side window panels (Figures 1 and 2). The dry pass-through (DPT) (Figure 3) is an air plenum passing through one of the window panels. The plenum is outfitted with a door on the inside, and a door on the outside, with a mechanical interlock system to ensure that both doors cannot be unintentionally opened at the same time, which might allow microbial contaminants to escape. The DPT contains a steam port and a drain for decontamination of the pass-through after each use, and condensate water is routed to the inside of the containment. The decontamination process of the DPT is independent of the decontamination process of the entire containment. This DPT was installed at an angle to facilitate the movement of materials into the containment. Also, a special stainless steel transfer container (0.6 m x 0.3 m x 0.3 m high) was designed (not shown) with one end door for loading inoculated produce through the DPT. The transfer container is filled with produce under a biosafety cabinet and then loaded into the DPT. The end door of the

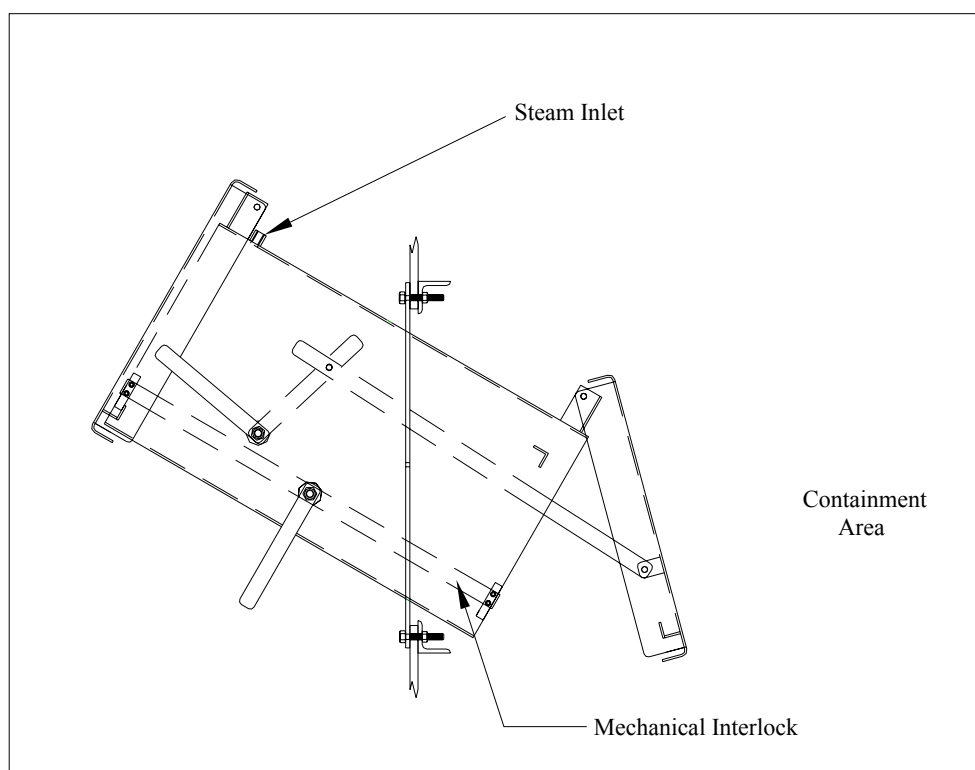
box is then unlatched, but is held closed by the latched inside door of the DPT. Next, the outside door of the pass-through is latched, allowing the inside door to be slowly opened, and allowing the produce to roll gently into the process equipment inside the containment.

The liquid pass-through (LPT) (Figure 4) is a tank divided by a wall, and is filled with approximately 90 liters of 500 ppm chlorine solution to prevent the escape of aerosols from the inside of the containment. It should be noted that the liquid level in the tank is higher on the inside of the containment than on the outside, owing to the vacuum inside. From the inside of the containment, samples are sealed in two Ziploc storage bags, and are submerged and held for 30 seconds in the chlorine solution, passed under the wall, and lifted out of the liquid on the outside of the containment. An integral collar made of square tubing surrounds the stainless steel tank and forms the surface for mating to the window. The structural angles on the inside surface of the windows help to complete the seal and carry the weight of the tank to the frame of the containment. The collar has a steam inlet at the top, and a condensate trap at the bottom, to help ensure that the interface is thoroughly heated during system decontamination. The tank's manual drain valve is inside the containment so that the liquid may be drained to the containment pans.

The pan shaped containment floor collects liquid

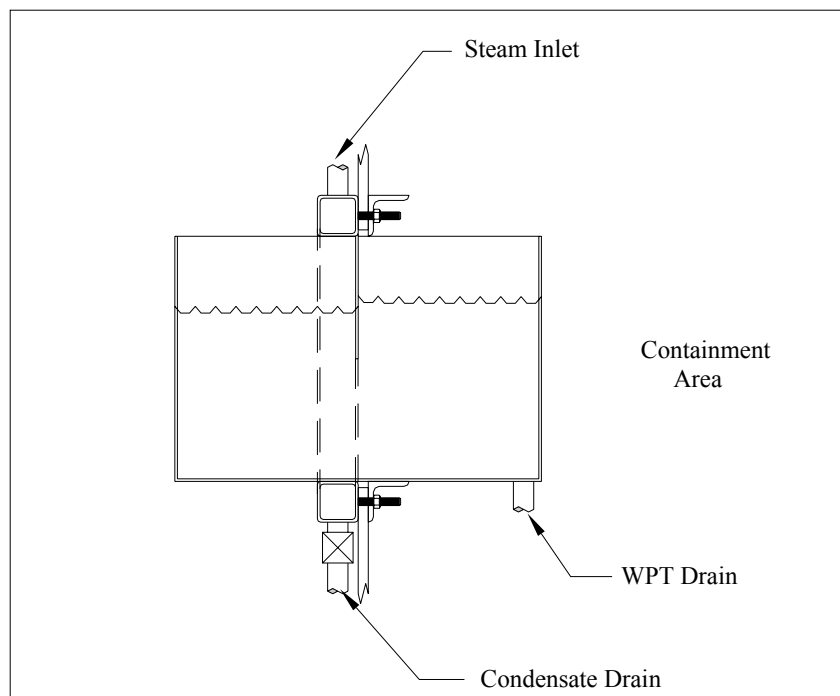
**Figure 3**

Dry pass-through: Top lever operates inside door and bottom lever operates interlock (details not shown) so that only one door can be open at a time. Some components exaggerated to show detail.



**Figure 4**

Liquid pass-through side view cross section. Liquid level is higher on inside because of negative pressure inside containment. Some components exaggerated to show detail.



and routes it to a drain (Figure 2). The liquid is a combination of condensate and water solutions from the process equipment that was splashed, spilled, or intentionally dumped. The drain liquids, and the drain itself, are decontaminated after each use of the containment by raising the liquid temperature through the use of a tube and shell heat exchanger (not shown) that is integral to the drain line.

During operation, the BSL-2 enhanced containment is kept under negative pressure using a regenerative blower (Figure 5). The path of air flow begins at the outside of the containment. Air enters the containment through any crevices in the structure, most notably where the windows are clamped to the frame. The filtered air then leaves the containment through a pipe attached to a top panel. The exhaust pipe leads to two sets of hydrophobic HEPA filters arranged in parallel, then to the blower. The discharged filtered air is ducted to the outside of the building. Each set of filters contains a coalescing 50.8 cm long Rigimesh Filter Element (Model #AB2RM7H4, Pall Corporation, East Hills, New York, USA) to reduce the amount of steam fog, followed by a 50.8 cm long hydrophobic PTFE (0.2  $\mu\text{m}$ ) HEPA filter (Model #AB2PFA7PVH4, Pall Corporation) to remove contaminants. The parallel nature of the filter sets allows for the use of multiple sets of filters to increase filtering capacity. When needed, each set can be isolated from the other set during operation by closing valves, decontaminated with steam, and then the filter housings opened to

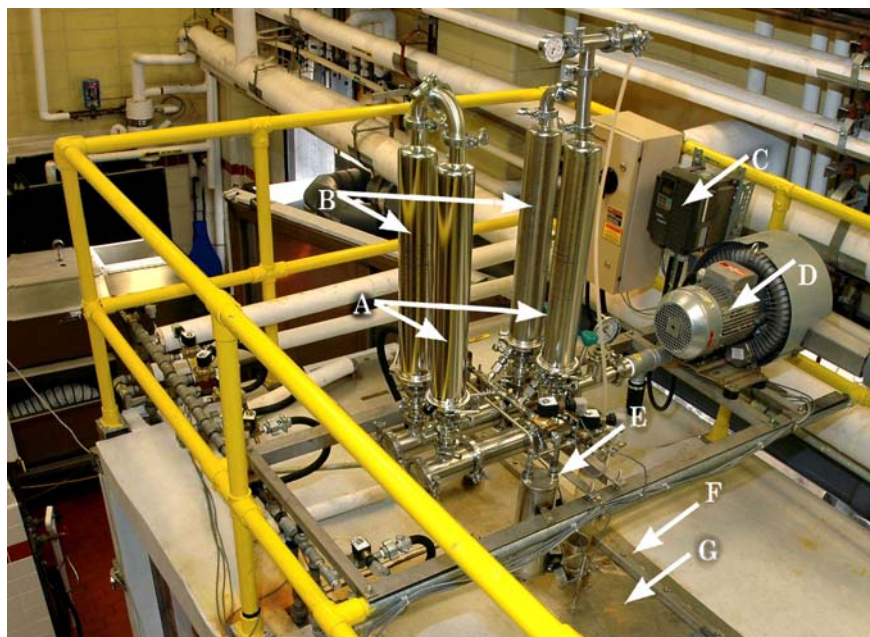
safely change filter elements.

Busch regenerative blower (Model #SB 0310 D2 HO EW XX, Dr. Ing. K. Busch GmbH, Maulburg, Germany) is intentionally installed downstream of the filters, because the high pressure side of the blower is above atmospheric pressure, which means the air must be filtered before reaching this point to avoid contamination leaks through any of the crevices. In other words, the entire system, from the containment through the filters, is under negative pressure to avoid such leaks of contamination. The regenerative blower is powered by an electric motor controlled by a GE-Fuji frequency modulation controller (Model #6KP112307X4B1, GE Industrial Systems, Salem, Virginia, USA) with feedback from a Magnelic Differential Pressure Indicating Transmitter Dwyer (Model #605-3, Dwyer Instruments, Inc., Michigan, Indiana, USA) in order to regulate the pressure inside the containment. As an added safeguard, this pressure indicating transmitter controls the steam valve; if the vacuum inside the containment falls to below 0.2 mm of Hg, the valve is closed.

The BSL-2 enhanced containment system and the processing equipment are simultaneously controlled by a personal desktop computer (Figure 2) with Lab View 7.1 software (National Instruments, Houston, Texas, USA). The computer system, which can be controlled remotely through a local area network, is located next to the containment unit.

**Figure 5**

Containment Exhaust System: A - Coalescing Filter; B - HEPA Filters; C - Variable Frequency Drive; D - Regenerative Blower; E - Condensate Trap; F - Linear Clamping Bar; G - Removable Plate



### Temperature Monitoring

Air and water temperatures at various locations within the containment and DPT were monitored and recorded using a data acquisition system consisting of Resistance Temperature Detectors (RTDs), smart transmitters (Model #AI-100 R-2, Accuteck, Hudson, Massachusetts, USA) with a 4-20 mA output, one data acquisition card (Model #NI-PCI-6024E, National Instruments). This system was calibrated with a high accuracy digital thermometer (Model #DP97, Omega Engineering, Stamford, Connecticut, USA).

Surface temperatures were measured with non-reversible temperature indicator strips Thermax 6ML-3 (Thermographic Measurement Ltd., Glenview, Illinois, USA), having a range of 65-93°C, with approximately 6°C increments, and an accuracy of  $\pm 1^\circ\text{C}$ , or with 30 AWG surface mounted fiberglass insulated thermocouples (Model E SA1XL-T-72, Omega Engineering).

### Containment and Decontamination Process

Produce processing equipment, such as the dip tank, which is an experimental pilot scale produce cleaning device designed by the ERRC-PSU team that has a capacity of 235 liters, and is capable of operating at temperatures between 4-95°C, is placed inside the containment; the doors are closed with the gasket steam pressurized. The regenerative blower is then started. From this point, the profile of a typical cycle consisting of five main phases is shown in Figure 6.

*Containment Phase—Section “A”:* The regenerative blower creates a negative pressure (typically 0.25 cm water

pressure) inside the containment that induces an inward airflow to ensure that the flow is from outside the containment to inside the containment through any crevices, or other openings in the containment. This air flow then exits the containment through the HEPA filters (Figure 5), through the regenerative blower, out of the building and through a local exhaust blower.

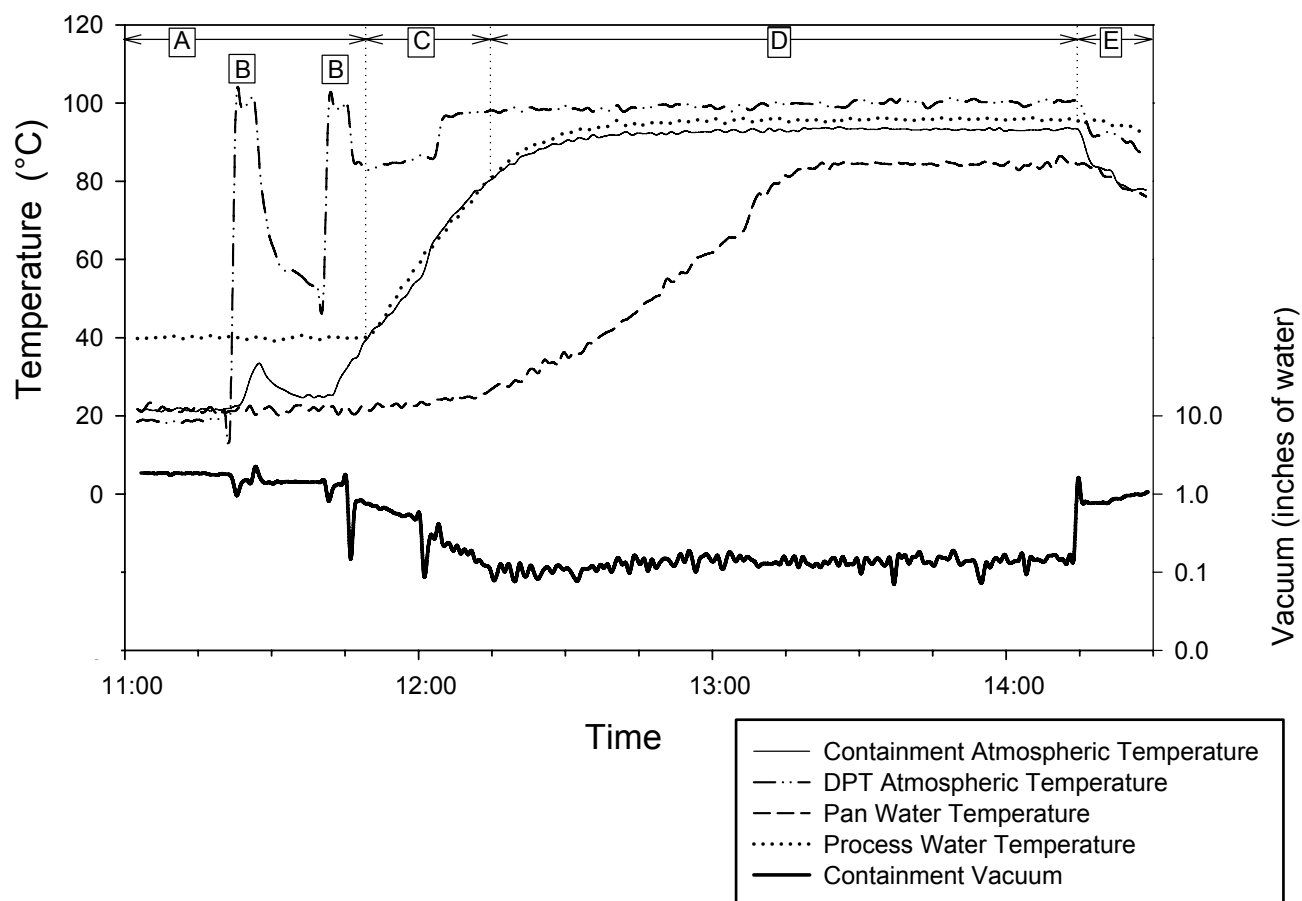
*DPT Decontamination Phase—Section “B”:* While produce treatments are being remotely executed inside the containment, test materials can be sequentially added to the processing equipment by using the DPT. The DPT and the transfer container are routinely decontaminated independently of the containment by injecting atmospheric steam into the DPT between runs. This ensures that any airborne microorganisms will not escape the containment into the room when the DPT is opened for loading the next produce sample. Produce samples are removed from the containment through the LPT. Produce not needed as samples may remain in the containment through the decontamination cycle.

*Containment Heating Phase—Section “C”:* Once the experimental trial is complete, the containment is heated by pressurizing the frame with steam and injecting steam from the containment ceiling into the containment atmosphere to achieve a minimum atmospheric temperature of 80°C, as measured at the lower right-hand corner of the containment directly below the DPT (Figure 2).

*Dwell Phase—Section “D”:* Once the containment temperature reaches 80°C, the program maintains this temperature, or higher, for two hours, which was experimentally determined to be effective for complete inactivation

**Figure 6**

Typical temperature profile of BSL-2 containment during produce processing and decontamination cycles. A - Containment Phase; B - Dry Pass-Through Decontamination Phase; C - Containment Heating Phase; D - Containment Decontamination Phase; E - Containment Cool Down Phase



of all *Escherichia coli* ATCC 25922 cells inoculated on the surfaces of the containment and processing equipment within the containment (data not shown). During this time, steam is continuously injected, unmodulated, into the containment, and the blower is continuously operated to maintain the negative pressure. If during the dwell period, atmospheric temperature falls below 80°C, the dwell period would be restarted. Because steam flow is not modulated during the dwell period, the atmospheric temperature continues to rise and typically increases to 90°C or above.

**Containment Cool-down Phase—Section “E”:** Steam flow is stopped, but the blower continues to operate. After fifteen minutes, most of the hot humid air from the containment is removed and the doors may be opened. An additional cooling period of at least 15 minutes is usually desired to avoid potential burns to personnel.

#### Validation of BSL-2 Enhanced Containment

Validation of the BSL-2 enhanced containment was completed under three different conditions using pathogenic *Salmonella* and a non-pathogenic *Escherichia coli*. The

validation conditions included containment of aerosol generation and inactivation, decontamination of inoculated stainless steel coupons (SSCs), and validation of a pilot-scale fruit treatment process.

*Bacterial strains, maintenance, growth conditions, and inoculum development:* *Salmonella poona* RM 2350 (California Department of Health Services 00A3563), a clinical isolate associated with a cantaloupe outbreak, was obtained from Dr. William Fett (USDA-ARS-ERRC, Wyndmoor, Pennsylvania, USA). *E. coli* ATCC 25922, a non-pathogenic surrogate for *S. poona* (Eblen et al., 2005), was obtained from American Type Culture Collection (Manassas, Virginia, USA). Stock cultures were stored in tryptic soy broth (TSB; BBL/Difco, Sparks, Maryland, USA) containing 20% glycerol at -80°C. Working stocks were maintained on tryptic soy agar (TSA; BBL/Difco) slants containing 0.6% yeast extract (YE; BBL/Difco) stored at 4°C for two to four weeks. A loop full of culture from a TSA-YE slant was transferred into 10 ml of TSB and allowed to grow for approximately eight hours at 35°C. This culture was then used to inoculate two liters of the same growth medium at 0.01 % (v/v) level. The

culture was allowed to grow to stationary phase at 35°C for 18 hours, and was used for inoculation of the BSL-2 containment, SSCs, or cantaloupes. The culture purity of non-pathogenic *E. coli* was assessed using the selective media Cefixime-Tellurite-Sorbitol MacConkey agar (CTS-MAC; BBL/Difco), MAC, and Eosin Methylene Blue agar (EMB; BBL/Difco). The culture purity of *S. poona* was checked using the selective medium Xylose-Lysine-Turgitol-4 agar (XLT-4; BBL/Difco). Also, cultures were examined serologically for identity, using commercial latex agglutination tests (Eblen et al., 2005).

*Validation of aerosol contamination and decontamination of BSL-2 enhanced containment using atmospheric steam treatment:* An important function of the BSL-2 enhanced containment is to contain aerosols contaminated with microorganisms, in particular, human pathogens. To validate the containment of aerosols generated inside the BSL-2 enhanced containment, a TSB grown culture of non-pathogenic *E. coli* was sprayed on all reachable surfaces throughout the containment interior using a Hudson Industry pressurized sprayer (H.D. Hudson Manufacturing Company, Chicago, Illinois, USA) with a brass Tee Jet 8004E nozzle tip (Spraying Systems, Co., Wheaton, Illinois, USA). The culture, contained in two 1-liter Pyrex bottles, and the pressurized sprayer were brought into the containment. The two doors to the containment were closed, with the gaskets steam pressurized, and the regenerative blower creating a negative pressure in the containment before spraying. Spraying was done from outside the containment using the glove ports near the LPT (Figure 1) to transfer the culture into the sprayer and to operate the sprayer to create the aerosol. Samples of the culture were taken from the exit of the spray nozzle and tested to ensure viability at the start of each experiment. These, and future samples, were brought out of the containment in Ziploc storage bags through the LPT, as described above, sealed in a leak proof plastic container, and transported to a microbiological laboratory for analysis.

Aerosol testing was completed using a SAS-super100 air sampler (Bioscience International, Rockville, Maryland, USA). Five hundred liters of air were drawn over a TSA plate. During spraying, several air samples were taken outside the containment in areas near the containment doors, near the gaskets joining the two containment modules, and at the regenerative blower outlet. To collect samples inside the containment, the air sampler was then encased in two Ziploc storage bags and passed into the containment through the LPT. Beginning one hour after spraying, air samples were taken inside the containment. After the removal of the sampler, the decontamination cycle was started, incorporating a holding time of two hours, once the atmospheric temperature reached 80°C. After a cool-down phase of 15 minutes, FS Foam Chlor (Zep Manufacturing Product #244424; Atlanta, Georgia, USA) was sprayed along the exterior of the doorway gaskets for added safety before opening the doors of the con-

tainment. Air samples were again taken inside and outside of the containment.

In a similar trial, the efficacy of the decontamination cycle to decontaminate surfaces of the containment and its components after being sprayed with *E. coli* suspension, was examined. Before the containment was closed, sample sites were swabbed to ensure that there was no *E. coli* before spraying with that culture. Whirl-Pak® Speci-Sponge® environmental surface sampling bags (NASCO, Fort Atkinson, Wisconsin, USA), hydrated with 25ml of 0.1% peptone water (PW) (BBL/Difco) were used to swab 8 x 8 cm sections of the window, door gasket, door, ceiling, and parts of the dip tank; a sample was taken of the floor pan water to recover contaminants. The same areas were sampled after the decontamination cycle to recover possible surviving *E. coli*. During spraying, swabs of the door gasket, exterior to the containment, were taken to determine whether *E. coli* had leaked out around the gasket. One hour after spraying with the containment at normal room temperatures, swabs of the interior window surfaces, where the operator could reach using the glove port near the LPT, were taken to confirm that viable *E. coli* contamination was present on the containment's interior surfaces prior to the decontamination process. All samples were enumerated as described below.

*Validation of decontamination of inoculated stainless steel coupons using atmospheric steam treatment:* Thirty 304 series SSCs (2 cm x 8 cm x 0.15 cm) with a #4 sanitary finish were inoculated with *E. coli* and *S. poona* in separate trials, by immersion in TSB-grown culture for five minutes, and were allowed to dry for two hours in a biological safety cabinet (BSC) until visibly dry. Inoculated SSCs were stored individually in a 50 ml sterile polypropylene conical tube for 24 hours at room temperature (RT = 19 ± 1°C) prior to treatment. The inoculated coupons were then transported to the BSL-2 facility in a sealed plastic container and were used to validate the process of using atmospheric steam for the decontamination of the BSL-2 enhanced containment. Twenty-seven inoculated SSCs were hung using previously installed hooks in the containment. Nine coupons were mounted by the left door, nine coupons were mounted in the center of the containment and nine coupons were mounted by the right door. The test coupons were equally spaced in the horizontal and vertical planes. After the coupons were mounted, TSB-grown culture of *E. coli* was added to the floor pan and dip tank water, to give a final concentration of 3 log CFU/ml to determine if this water reached a high enough temperature to inactivate the bacteria. Once the test coupons were installed and the water was inoculated, the containment doors were securely closed. To simulate a produce disinfection trial, water in the dip tank was circulated for 15 minutes at the maximum flow rate. At the same time, two air samples were taken near the regenerative blower where the HEPA filtered air was exhausted. TSA plates in the air sampler were each exposed

to 500 liters of this air during the five-minute sampling time. Once the simulated disinfection treatment was completed, the dip tank water and the containment were heated simultaneously to a minimum of 80°C at which time the containment's two-hour holding time at  $\geq 80^\circ\text{C}$  began, followed by the 15-minute cooling phase. The coupons were then collected and placed into individual sterile 50 ml polypropylene conical tubes containing 30 ml of TSB and sterile glass beads. Water samples were collected from the floor pan and the dip tank. All samples were transported in sealed plastic containers marked with the universal biohazard symbol for microbial analysis, as described below, to a microbiological laboratory. This validation protocol using SSCs was repeated three times.

*Validation of pilot-scale process treatment of cantaloupes using the BSL-2 containment:* Experiments with cantaloupes were used to validate a pilot-scale thermal surface pasteurization process (Annous et al., 2004) applied in the dip tank inside the containment. Cantaloupes, weighing between 1134 and 1764 grams and free of decay and punctures, were obtained from local suppliers and grocery stores and stored at 4°C for no more than two days prior to use. These cantaloupes were inoculated with *E. coli* and *S. poona* in separate trials using the dip method (Annous et al., 2004). Briefly, TSB-grown culture of *E. coli* or *S. poona* were centrifuged down at  $6740 \times g$  for 20 minutes, washed once with 400 ml sterile deionized water, and suspended in two liters of sterile deionized water to give a final cell concentration of approximately  $9 \log \text{CFU/ml}$ . This inoculum was used to inoculate cantaloupes as described below. The inoculum was stable in deionized water for at least 24 hours, as seen by the recovery of similar microbial cell densities at time zero, four, and 24 hours (Annous et al., 2004). The cantaloupes were submerged individually in inoculum for five minutes, then drained and allowed to air dry on an absorbent paper for two hours (one hour on each side) in a biosafety cabinet. The cantaloupes were then stored in plastic tubs lined with an absorbent paper at room temperature for 24 hours prior to treatment. The inoculated cantaloupes were then transported to the BSL-2 facility in a sealed plastic container (BMBL, 1999), and loaded into the transfer container in a class II A2 BSC before being introduced into the DPT through which they entered the closed containment and were released manually from outside the containment into the dip tank. By operations of the dip tank, triplicate samples, each consisting of three cantaloupes, were totally immersed in the untreated tap water at RT, or 76°C for three minutes, immediately sealed in plastic bags, and submerged in a water-ice bath for five minutes. Most of the air in the plastic bag was removed prior to sealing to minimize any effect on heat transfer during the cooling process. The cantaloupes were then analyzed for residual surface microbial population using the whole rind method of sampling (Annous et al., 2004).

Between the two temperature treatments, the transfer

container and pass-through were decontaminated with steam for five minutes at or above 93°C, and swabs were taken to verify that this operational process decontaminated the DPT. Additional swabs were taken at the DPT door, bottom of the transfer container, the DPT's internal latching mechanism, and the ramp used to convey the cantaloupes from the DPT to the dip feed. After completion of the cantaloupe decontamination treatments, the containment was decontaminated using the decontamination cycle described above. The dip tank entry and exit ramps were swabbed after the containment decontamination. Water samples from the dip tank and floor pan were also collected and tested for the presence of test microorganism. All samples were enumerated as described below.

### Enumeration and Recovery of Bacteria

At the conclusion of trials to validate the performance of the containment (including its use in pilot scale experiments and subsequent decontamination of the system), swab samples, test coupons, water samples, cantaloupes and TSA plates used with air sampling, were removed from the containment area and transported to the microbiology laboratory for analysis in sealed containers as described above. TSA was used for enumeration of total aerobic microorganisms, and as a recovery medium for injured cells of *E. coli* and *S. poona*. Enumeration of uninjured *E. coli* and *S. poona* populations was done using the selective media MAC and XLT-4, respectively. Recovery medium (TSA) plates were incubated at 35°C for two hours to allow for recovery of injured *E. coli* and *S. poona* cells and then overlaid with the appropriate selective medium. All plates were incubated for 24 hours at 35°C, and the resultant colonies were counted manually. Cell densities were reported as  $\log \text{CFU per cm}^2$  or per ml.

*SSCs:* Treated and non-treated SSCs in 50 ml centrifuge tubes containing 30 ml TSB and sterile glass beads were vortexed for one minute. TSB samples were serially diluted and plated on MAC, or XLT-4, and on TSA with a MAC, or XLT-4 overlay. The remaining samples were incubated overnight at 35°C for enrichment and recovery of cells present in numbers below the detection limit, and were plated on MAC or XLT-4. Typical colonies growing on MAC, or XLT-4 were checked for identity using the procedures described above.

*Cantaloupes:* The whole rind (flesh-free) of a cantaloupe was removed using a sterile Muro Peel-All Fruit Peeler CP-44 (Muro Corporation, Tokyo, Japan), placed in a sterile one-liter glass blender jar (Waring Products, Torrington, Connecticut, USA), combined with four equal volumes (w/v) of PW, and blended at medium speed for one minute with a commercial blender (Waring Blender, Model 51BL31). The resulting blend was filtered through a filter bag (Spiral Biotech, Bethesda, Maryland, USA), and duplicate 10 ml volumes were transferred to sterile tubes. Filtrates were diluted in PW as necessary, and surface plated on the appropriate growth medium as

described above.

**Water Samples:** Water samples were plated on MAC, or XLT-4 and TSA, with MAC or XLT-4 overlay as described above. Enrichment of water samples was completed by mixing 10 ml of the water sample with 10 ml of sterile double strength TSB, and was incubated at 35°C overnight. Enriched samples were plated on MAC or XLT-4. Typical colonies growing on MAC, or XLT-4 were checked for identity using the procedures described above.

**Air Samples:** TSA plates used with air sampling were overlaid with MAC, or XLT-4 following incubation at 35°C for two hours as described above. Typical colonies recovered on TSA overlaid with MAC or XLT-4 were checked for identity using the procedures described above.

**Swab Samples:** Sponges were processed using a Stomacher 400 circulator (Seward, England) at 230 rpm for one minute. The samples were serially diluted and plated on MAC, or XLT-4 and on TSA with a MAC or XLT-4 overlay. The remaining samples were incubated overnight at 35°C for enrichment and recovery of cells present in numbers below the detection limit, and were plated on MAC or XLT-4. Typical colonies growing on MAC, or XLT-4 were checked for identity using the procedures described above.

### Scanning Electron Microscope (SEM)

SSCs were examined following inoculation for visualization of attachment and biofilm formation by *E. coli* and *S. poona*. Duplicate samples were fixed by immersion in 2.5% glutaraldehyde-0.1 M imidazole buffer solution (pH=7.0) for two hours and stored in sealed tubes at room temperature until further processing. Fixed samples were then washed in the buffer, dehydrated in a graded series of ethanol (50%, 80%, then absolute) and critical-point-dried by liquid carbon dioxide. Samples were coated with a thin layer of gold by DC sputtering. Digital images were collected in the secondary electron imaging mode of a SEM Model Quanta 200 (FEI, Hillsboro, Oregon, USA).

### Statistical Analysis

Analysis of variance with individual contrasts and Bonferroni T-tests were used to determine significant differences ( $p < 0.05$ ) among population means in response to treatments. Unless otherwise indicated, the level of significance used was  $p = 0.05$ . All statistical analyses and calculations of means and standard deviations were performed using SAS/STAT software (SAS Institute Inc., Cary, North Carolina, USA).

### Results and Discussion

The decontamination dwell period was set at 120 minutes, and the data were extracted in 15-minute intervals starting at 45 minutes into the decontamination cycle. The temperatures of the wastewater and atmospheric air at a bottom corner of the containment, and at an additional location directly underneath the DPT (coldest point of the containment), were measured with the instrumentation described above. The temperature of a motor core was measured with a surface mounted thermocouple at the rotor of a stationary 375 W wash down motor (Leeson, Grafton, Wisconsin, USA). The motor was located on the containment floor directly under the DPT next to the RTD. Resulting temperature data are shown in Table 1. Data show that a temperature in excess of 80°C was maintained for over 100 minutes. This time-temperature combination was previously shown to be sufficient for total inactivation of *E. coli* ATCC 25922 cells applied to surfaces inside the containment and processing equipment placed within the containment system (data not shown).

With the air temperature profile well established, the surface temperature of the internal components was determined for various dwell periods. Twenty-seven test coupons with temperature indicator strips were adhered to the SSCs surfaces. These SSCs were mounted adjacent to the inoculated SSCs. In addition, similar temperature indicators strips were mounted onto high mass surfaces, such as pumps, gear motors, and the floor pans. The read-

**Table 1**

Temperature profiles of various locations within the containment and the water inside the processing tank collected during the dwell phase, following steam injection heating of the atmosphere to 80°C (Section D of Figure 6).

	Temperature (°C)						
	0 minutes	45 minutes	60 minutes	75 minutes	90 minutes	105 minutes	120 minutes
Dry Pass-Through (DPT)	98.0	99.3	100.4	100.0	99.2	100.9	99.3
Wastewater floor (pan)	27.6	76.9	89.6	92.6	92.9	93.1	94.2
Containment atmosphere (Opposite DPT)	80.4	87.0	87.1	87.4	88.0	87.6	88.6
Containment atmosphere (Under DPT)	77.1	84.9	85.0	85.4	87.9	86.0	86.0
375 W (1/2) Hp Motor (Measured at rotor)	58.7	89.6	90.7	91.6	93.7	93.0	93.8

ing of each surface temperature profile was recorded following 60- and 120-minute dwell periods cycle (Table 2). With only a 60-minute dwell period, the highest temperature achieved at the coldest location of the containment was 71°C. Even though this temperature is sufficient to deactivate the bacterial species of concern, the possibility exists of cooler sections inside the containment where these bacterial cells might survive. The goal was to have the containment at a temperature significantly above the kill point of the bacteria, which should guarantee safe entry into the containment. The temperature of containment surfaces were equilibrated at or above 93°C as confirmed by the indicator strips at the end of a 120-minute dwell period.

The surface temperature of DPT was validated by directly attaching indicator strips to the DPT surfaces. One strip was placed inside the transport box; another strip was placed in the mechanism area, and the final strip was placed in the actual pass-through area. Steam was applied to the pass-through for five minutes, while recording the atmospheric temperature profile inside the pass-through. The indicator strips reached their maximum temperature of 93°C, while the atmospheric temperature reached 100°C in two minutes. Air and surface temperature profiles of the containment and DPT following the 120-minute dwell period (Tables 1 and 2) along with the microbiological data, indicated that conditions used for the decontamination of the containment ensured total inactivation of vegetative bacterial cells on the surfaces and in the atmosphere of the containment.

### Validation of Aerosol Containment and Decontamination of BSL-2 Containment Using Atmospheric Steam Treatment

TSB grown culture (2 L) of non-pathogenic *E. coli* ATCC 25922 containing 9 log CFU/ml was sprayed on all reachable surfaces of the containment. Besides contaminating the surfaces, spraying of the culture generated

aerosols inside the containment for the purpose of investigating the ability of the containment to contain any potentially airborne microbial cells during the processing of produce inoculated with human pathogens. Cell concentrations of *E. coli* one hour following spraying on containment and equipment surfaces, and in water on the containment floor, were an average of 7 log CFU per cm<sup>2</sup> or ml (data not shown). Concentrations of *E. coli* cells in air samples collected from inside the containment 15 and 60 minutes after spraying, were 3 and 1 log CFU/sample, respectively. All water, surface and air samples (including enrichment) collected from inside and outside the containment prior to spraying were negative for the presence of *E. coli*. Environmental samples collected from outside the containment during spraying of the culture were negative for *E. coli* cells, with some exceptions, which are listed in Table 3. In two incidents, a door gasket leaked during spraying. The first incident was due to the lodging of a foreign object between the gasket and the door. The problem was corrected and the Standard Operating Protocols were updated to include inspection and cleaning of these gaskets. In the second incident, the leak was attributed to a rough weld joint at the interface. This weld joint was ground smooth and the remaining joints were inspected and smoothed as necessary. The integrity of the gaskets was re-validated following correction of these problems. Results showed that the gaskets were able to contain the culture and did not allow any leakage to the outside of the containment.

Another potential source of airborne contamination could result from opening the containment door when agent containing aerosols are present inside. It was hypothesized that when the door was opened, a pressure gradient would develop that would transport the bacteria from inside the containment to the exterior work space. To test this theory, investigators sprayed the interior of the containment. After allowing 15 minutes for the aerosol to settle (approximately two containment air ex-

**Table 2**

Surface temperatures of various locations within the containment following a 60- and 120-minute decontamination cycle as measured by indicator strips.

	Temperature (°C)					
	60 minutes			120 minutes		
	Minimum	Maximum	Average	Minimum	Maximum	Average
Dry Pass-Through End of Containment	71	88	82	93	93	93
Middle of Containment	77	82	83	93	93	93
Liquid Pass-Through End of Containment	82	93	86	93	93	93
Dry Pass-Through	93	93	93	93	93	93
Conveyor Motor	82	82	82	93	93	93
Pump Motor	82	82	82	93	93	93
Floor Pans	ND	ND	ND	82	93	91

ND = Not Determined

**Table 3**

Recovery of *Escherichia coli* ATCC 25922 cells from air and surface samples collected from containment and equipment following a steam decontamination cycle.

	TSA with MAC Overlay						MAC				
	Air	Window	Equip.	Metal Surfaces	Gaskets	Pan Water	Window	Equip.	Metal Surfaces	Gaskets	Pan Water
Feb. 15, 2005	NG <sup>a</sup>	NG	NG	NG	NG <sup>b</sup>	NG <sup>c</sup>	NG	NG	NG	NG <sup>b</sup>	NG <sup>c</sup>
Feb. 1, 2005	NG	NG	NG	NG	NG	NG <sup>d</sup>	NG	NG	NG	NG	NG <sup>d</sup>
Oct. 4, 2004	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG
Sept. 27, 2004	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG
May 5, 2004	NG <sup>e</sup>	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG
April 21, 2004	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG
April 20, 2004	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG
April 13, 2004	NG <sup>f</sup>	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG
Feb. 12, 2004	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG

<sup>a</sup> NG = No growth was detected with all samples tested (enriched and non-enriched samples).

<sup>b</sup> Bottom right-hand corner of the seal tested positive for *Escherichia coli* (4.7 CFU/ml) during spraying. No cells were recovered following the decontamination cycle. The problem was attributed to a rough weld joint. This weld joint was ground smooth and the remaining joints were inspected and smoothed as necessary.

<sup>c</sup> Following the decontamination cycle, water samples collected from the pan inside the containment were negative for *Escherichia coli*, but the water collected from the outside manifold was positive for *Escherichia coli* (>4 log CFU/ml). It was determined that the water in the outside manifold did not reach the temperature required to thermal inactivate the bacteria. A heat exchanger tube in tube heat exchanger was designed and installed. After the modification, the temperature inside the manifold now approaches 100°C.

<sup>d</sup> Due to a control failure, the containment reached and maintained a temperature of 80°C or above for 18 minutes of the 120 minutes. The problem was caused by a programming error, which was later corrected.

<sup>e</sup> A door gasket leak caused bacteria to become airborne. Air samples collected from outside of the containment were positive for *E. coli*. The problem was a foreign object between the gasket and the door frame. The problem was corrected and the Standard Operating Protocols were updated to include inspection and clean-up of these gaskets.

<sup>f</sup> Fifteen minutes following spraying, which allowed 15 minutes for the aerosols to settle, the door of the containment was opened and air samples were collected from inside and outside of the containment. These air samples tested positive for *Escherichia coli* (1-3 log CFU/air sample). Air samples collected from outside the containment prior to spraying, during spraying, and 24 hours post spraying, were negative for the presence of this microorganism.

changes), the door of the containment was opened and air samples were collected from inside and outside of the containment. These air samples tested positive for *E. coli* (1-3 log CFU/sample). Air samples collected from outside the containment prior to spraying, during spraying, and 24 hours post spraying, were negative for the presence of this microorganism. This indicated that the containment should be decontaminated prior to opening the doors to avoid the release of any potentially airborne contaminants into the environment.

All air samples, surface swab samples, and water samples, including enriched samples collected from inside the containment following the decontamination treatment with atmospheric steam, were negative for the presence *E. coli* cells (Table 3). The containment contains two modules and each module contains two floor pans. These pans are manifolded together with a pipe outside containment and water samples collected from the manifold were positive for *E. coli* (>4 log CFU/ml) because of insufficient heating. Therefore, a heat exchanger was installed to raise the waste water temperature to nearly 100°C. To

validate this design, a TSB grown culture of *E. coli* was added to the water on the floor pan water to give a final cell concentration of 3 log CFU/ml. Water samples collected from the inside of the containment and the outside manifold following the decontamination cycle were negative for the presence of *E. coli* cells. This indicated that following decontamination, the manifold was free of any potential contaminant as a result of processing produce inoculated with human pathogens.

The data presented in Table 3 indicated that the containment unit prevented the airborne contaminants generated during the processing of produce inoculated with human pathogens from entering into the pilot plant facility. Also, atmospheric steam was capable of completely inactivating *E. coli* cells in the air and on the containment surfaces after aerosol generating, or other experiments, without the risk of exposing the operator to pathogens. This allows for opening of the containment for replacing processing lines without the risk of exposing the operator to potential airborne foodborne pathogens.

### Validation of the Use of Atmospheric Steam for Inactivation of Bacterial Cells in Biofilm

Decontamination of inoculated SSCs was conducted to validate the ability of atmospheric steam to inactivate microbial cells present in a biofilm. Biofilm formation by *E. coli* ATCC 25922 and *S. poona* RM 2350 on produce surfaces was reported to be responsible for increased resistance to aqueous sanitizing washes (Annous et al., 2004; Annous et al., 2005a; Annous et al., 2005b). Although atmospheric steam inactivated *E. coli* cells on inoculated surfaces of the containment following spraying, cells were not allowed enough time for strong attachment and biofilm formation. Therefore, SSCs were inoculated and dried for 24 hours at RT to allow for strong attachment and biofilm formation on the surfaces. Attachment and biofilm formation by *E. coli* (Figure 7) and *S. poona* (Figure 8) on SSCs' surfaces were established using SEM. Thirty SSCs, inoculated with *E. coli* or *S. poona* (Table 4), were attached at different locations within the containment and the DPT. All SSCs, including enriched samples, were negative for the presence of *E. coli* or *S. poona* cells following the decontamination cycle of the containment using atmospheric steam (Table 4). These results

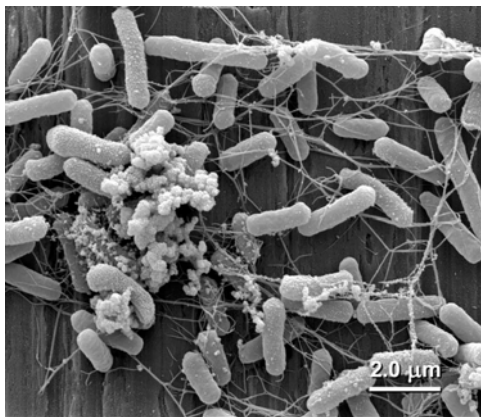
indicated that atmospheric steam was capable of inactivating cells that might exist in biofilms on the surfaces of the containment and/or equipment within. Also, this would indicate that any microbial contaminants on the surfaces of the containment modules, as a result of processing produce inoculated with human pathogens, would be inactivated by atmospheric steam as previously demonstrated.

### Validation of Pilot-Scale Process Treatment of Cantaloupes

Development and fabrication of the pilot-scale dip tank for washing produce or surface pasteurization was completed as part of this research project. Validation of the dip tank for surface pasteurization of cantaloupes inoculated with pathogenic *S. poona* RM 2350, or the non-pathogenic *E. coli* ATCC 25922 within the containment system, was carried out as described above. Surface pasteurization of cantaloupes at 76°C for three minutes resulted in significant reductions in *S. poona* (Table 5) and *E. coli* (Table 6) cell densities compared to the controls. Six of the nine cantaloupes inoculated with *S. poona*, and three of the nine cantaloupes inoculated with *E. coli*, showed no growth on selective and recovery medium.

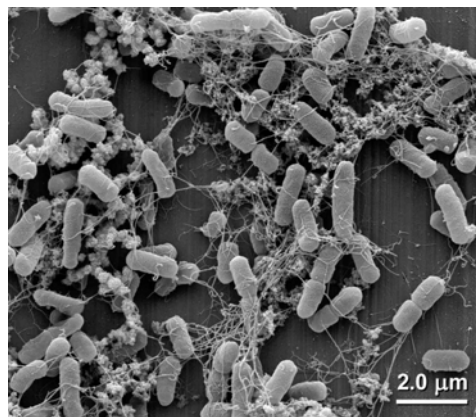
**Figure 7**

Scanning Electron microscopy image of biofilm formation by *Escherichia coli* ATCC 25922 cells on a stainless steel coupon.



**Figure 8**

Scanning Electron microscopy image of biofilm formation by *Salmonella poona* RM 2350 cells on a stainless steel coupon.



**Table 4**

Efficacy of decontamination process of containment on recovery of *Escherichia coli* ATCC 25922 and *Salmonella poona* RM 2350 cells from inoculated stainless steel coupons<sup>a</sup>.

Samples	<i>Escherichia coli</i> ATCC 25922 (log CFU/cm <sup>2</sup> )		<i>Salmonella poona</i> RM 2350 (log CFU/cm <sup>2</sup> )	
	MAC	TS with MAC overlay	XLT-4	TSA with XLT-4 overlay
Control	4.14 ± 0.53	4.70 ± 0.49	4.55 ± 0.26	5.78 ± 0.46
Decontamination Treatment	NG <sup>b</sup>	NG	NG	NG

<sup>a</sup> Results are reported as the mean ± standard deviation of three independent runs. Each run consisted of three different inoculated SSC.

<sup>b</sup> NG = No growth was detected with all samples tested including enriched samples.

**Table 5**

Efficacy of pilot-scale surface pasteurization (76°C) in decontaminating cantaloupes inoculated with *Salmonella poona* RM 2350<sup>a</sup>.

Treatment <sup>c</sup>	Residual population of <i>Salmonella poona</i> RM 2350 (log CFU/cm <sup>2</sup> ) <sup>b</sup>	
	XLT-4	TSA with XLT-4 overlay
2-h control	4.4 ± 0.4 A	5.0 ± 0.4 A
24-h control	6.0 ± 0.5 AB	6.8 ± 0.4 B
76°C for 3 minutes	0.8 ± 1.0 <sup>d</sup> C	1.0 ± 1.1 <sup>d</sup> C
Room temperature wash for 3 minutes	5.7 ± 0.6 AB	6.4 ± 0.6 AB
Decontamination Treatment	NG	NG

<sup>a</sup> *Salmonella poona* populations were enumerated on selective media (XLT-4) and recovery medium (TSA with XLT-4 overlay).

<sup>b</sup> Data are reported as the means ± standard deviations of three independent runs. Each run consisted of three cantaloupes. Means with the same letter are not significantly different ( $P < 0.05$ ).

<sup>c</sup> Cantaloupes were dip inoculated with *Salmonella poona* for five minutes, allowed to dry in a biosafety cabinet for two hours, and stored at room temperature for 24 hours prior to the washing treatment at 76°C or room temperature for three minutes.

<sup>d</sup> Although six of the nine cantaloupes tested showed no growth, 0.1 log CFU/cm<sup>2</sup> rind (minimum detection level) was used to determine the mean and standard deviation.

NG = No growth was detected with all samples tested including enriched samples.

**Table 6**

Efficacy of pilot-scale surface pasteurization (76°C) in decontaminating cantaloupes inoculated with *Escherichia coli* ATCC 25922<sup>a</sup>.

Treatment <sup>c</sup>	Residual population of <i>Escherichia coli</i> ATCC 25922 (log CFU/cm <sup>2</sup> ) <sup>b</sup>	
	MAC	TSA with MAC overlay
2-h control	5.0 ± 0.4 A	5.1 ± 0.4 A
24-h control	6.0 ± 0.5 A	6.2 ± 0.5 A
76°C for 3 minutes	1.4 ± 0.8 <sup>d</sup> B	1.5 ± 0.8 <sup>d</sup> B
Room temperature wash for 3 minutes	5.4 ± 0.6 A	5.7 ± 0.5 A
Decontamination Treatment	NG	NG

<sup>a</sup> *Escherichia coli* populations were enumerated on selective media (MAC) and recovery medium (TSA with MAC overlay).

<sup>b</sup> Data are reported as the means ± standard deviations of three independent runs. Each run consisted of three cantaloupes. Means with the same letter are not significantly different ( $P < 0.05$ ).

<sup>c</sup> Cantaloupes were dip inoculated with *Escherichia coli* for five minutes, allowed to dry in a biosafety cabinet for two hours, and stored at room temperature for 24 hours prior to the washing treatment at 76°C, or room temperature for three minutes.

<sup>d</sup> Although three of nine cantaloupes tested showed no growth, 0.1 log CFU/cm<sup>2</sup> rind (minimum detection level) was used to determine the mean and standard deviation.

NG = No growth was detected with all samples tested including enriched samples.

There was no significant difference between the controls and the washing treatment at room temperature for three minutes (Tables 5 and 6). This indicated that washing with water at room temperature was not able to dislodge, or remove cells of either bacterium attached to the surface of the cantaloupe. This resistance to water wash could be due to attachment of the cells to inaccessible sites on the rind of the cantaloupe and biofilm formation (Annous et al., 2004; Annous et al., 2005a; Annous et al., 2005b; Solomon et al., 2006). These results indicated that both microorganisms were thermally inactivated by this surface

pasteurization treatment (Annous et al., 2004; Solomon et al., 2006). There was no significant difference between recoveries of *S. poona* (Table 5) or *E. coli* (Table 6) cells on selective and recovery media, indicating that injury was not a factor during surface pasteurization treatments. Therefore, surface pasteurization treatment of cantaloupe can decrease the risk of foodborne illnesses.

Following completion of all processing trials, a 120-minute containment decontamination cycle was completed. The interior surfaces of the containment modules and the DPT, as well as water samples from the dip tank

and pan drains were collected for analysis. All samples, including enriched samples, were negative for the presence of *E. coli* or *S. poona* cells following the decontamination cycle of the containment using atmospheric steam. These results indicate that processing inoculated produce within the containment can be conducted without contaminating surrounding work areas, and that the decontamination of equipment and protocols developed are effective.

## Conclusions

This manuscript contains descriptions of the design, fabrication and validation of BSL-2 enhanced containment and pilot-scale surface pasteurization treatment of cantaloupes. The validation of a BSL-2 enhanced containment and processing treatment demonstrated that the procedures were fully successful with regards to containing and deactivating airborne, surface attached, and water suspended bacterial cells. The BSL-2 enhanced containment facility offers a unique opportunity to validate antibacterial interventions for decontamination of produce inoculated with human pathogens using pilot and industrial scale equipment. The research community now has a safe, workable, and proven engineering tool providing the ability to conduct pilot-plant scale decontamination studies of produce and equipment using human foodborne pathogens of interest, instead of surrogates.

Surface pasteurization treatment of cantaloupes using hot water meets the USFDA target of 5 log reduction in pathogen levels. The use of hot water as a method to decontaminate cantaloupe is more effective than various other washing and sanitizing treatments tested to date. The work presented in this paper demonstrates the utility of the containment system in validating surface pasteurization as an effective treatment that greatly reduce levels of *Salmonella* on the surface of inoculated cantaloupes.

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## Authors' Note

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