Self-contained chlorine dioxide generation and delivery pods for decontamination of floor drains[1]

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Primary Audience: commercial poultry processors and further processors, quality assurance personnel, poultry food safety researchers.

SUMMARY

Listeria monocytogenes can be present in poultry processing and further processing plants, where it can colonize floor drains. This is a concern as the potential exists for cross-contamination of product contact surfaces or even fully cooked product. Self-contained chlorine dioxide-generating and delivery pods were tested as a means to sanitize uninoculated floor drains and *L. monocytogenes*-inoculated model floor drains. Free-swimming and attached bacteria were enumerated in drains. Standing water and the inner surface of treated drains had significantly lower bacterial numbers than did untreated drains. *Listeria monocytogenes* numbers decreased by up to 8.7 log cfu/mL in standing water (>99.999999%) and 6.7 log cfu (>99.9999%) as attached cells. Commercially available chlorine dioxide pods may have practical utility for killing *L. monocytogenes* during periodic sanitization of floor drains in poultry processing facilities.

Key words: *Listeria monocytogenes*, floor drains, chlorine dioxide

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DESCRIPTION OF PROBLEM

Listeria monocytogenes is a human pathogen that has been associated with poultry meat products [1]. *Listeria monocytogenes* has been found to enter further processing facilities with raw product [2]. Once in the further processing plant, *L. monocytogenes* can become a long-term resident, colonize floor drains, and be detected even after clean-up and sanitation prior to starting a processing shift [2, 3]. This organism can readily form biofilms [4, 5, 6] or join existing biofilms on surfaces including the inner surface of floor drains. *Listeria monocytogenes* in floor drains is a concern because the potential for aerosolization of cells from drains has been documented [7, 8]. Such escape can result in contamination of product contact surfaces and even fully cooked ready-to-eat (**RTE**) meat product. Floor drain subtypes of *L. monocytogenes* have been detected on RTE meat and resulted in processors destroying contaminated product prior to distribution in order to assure the safety of consumers [9].

Chlorine dioxide (**ClO_2**) is an effective sanitizer that can be used in poultry processing [10, 11, 12]. We have found that ClO_2 can be effective to control human pathogens on carcasses

[1]Mention of trade names or commercial products in this publication is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture.

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during feather removal [11]. Other studies have reported that ClO_2 is an effective antimicrobial for killing bacteria, including *L. monocytogenes* attached to hard surfaces such as stainless steel and polyvinyl chloride (PVC) [13, 14). Generating large quantities of ClO_2 may be problematic in a processing plant. If the generation, storage or delivery system develops a leak, plant personnel may be at risk and the processing plant may have to be evacuated, resulting in a logistical and economic cost. A small-scale ClO_2 generation device that could place treatment directly into a floor drain for sanitation may prevent the need for large-scale generation on site. The objective of this study was to test commercially available, self-contained ClO_2 generating and delivery pods as a means to sanitize floor drains especially for the elimination of *L. monocytogenes*.

MATERIALS AND METHODS

Experimental Overview

Self-contained chlorine dioxide-generating and delivery pods were tested for disinfecting floor drains. Each pod is loaded with two precursor chemical components; when a pod is activated, the precursor chemicals are mixed and ClO_2 gas is created and released. Naturally contaminated drains were treated with pre-packed, commercially available pods [15] and sampled to determine the numbers of total aerobic bacteria attached to the inner surface of the drain pipe before and after treatment. Smaller, lab-scale model floor drains were constructed, inoculated with *Listeria monocytogenes* and treated with custom-packed, low-dose chlorine dioxide pods to measure efficacy against this specific pathogen.

Uninoculated Floor Drain Study

Floor drains located in 10 separate animal containment rooms were identified for testing efficacy of ClO_2 against naturally occurring drain microflora. In each of 2 replications, 5 floor drains were used; each floor drain was in a separate room and no drains were directly connected. Drains were standard PVC drain pipe soil stacks with an inner diameter of 10 cm (4 in). Drains ranged from 62.5 to 115 cm deep to the bottom of the trap and held approximately 20 cm of standing water. Maximum drain pipe volume from mouth to the bottom of the trap was approximately 9,027 cm^3.

Each drain was divided by a line into 2 vertical half cylinders, front and back. The front half cylinder in each pipe was sampled prior to treatment. Sampling was done by use of a sterile sponge [16] held in a sterile clamp on the end of a sample pole making it possible to rub the sponge along the inner surface of the front half cylinder at and below the water line to sample both free-swimming bacteria and those attached to the pipe's inner surface. Used sponges were re-placed into the associated sterile bags that were closed, covered with ice and transported to the laboratory for culture within 60 min. Following pre-treatment sampling, rubber adapters [17] were placed in the mouth of drains, pods [18] were activated according to manufacturer's instructions by twisting and shaking to introduce proprietary precursors to the mixing chambers and initiate generation of ClO_2 gas. One pod was placed face down in each adapter and allowed to emit gas into the drain for 4 h (total of 600 mg ClO_2). After treatment, pods were removed and the post-treated, back half cylinder of each drain pipe was sampled in the same way as the pre-treated half cylinder. Sponges from treated samples were re-placed in their respective sterile bags, covered with ice and transported to the laboratory for culture within 60 min.

Inoculated Model Drain Experiments

Model floor drains were constructed and inoculated as described previously [19]. Briefly, model drains made of PVC had one open end, and measured 10 cm wide and 15 cm deep (pipe volume 1,177 cm^3). Model drains were inoculated with *L. monocytogenes* in a 2-stage process as previously described for *L. innocua* [19]. A 24-h *L. monocytogenes* culture was grown at 35 °C on multiple plates of *Listeria*-selective agar – Oxford formulation (**LSA**) [20]. A cell suspension (10^8 cfu/mL of phosphate buffered saline, **PBS**) was prepared by removing colonies and adding to PBS. Cell suspension (325 mL) was added to each model drain and incubated

within for 2 h at 25 °C. Model drains were emptied, rinsed 3 times using a squeeze bottle filled with sterile water to remove unattached cells. Model drains were re-filled with 325 mL 1/10th strength brain heart infusion broth (**BHIB**) [21]. Model drains with $1/10^{th}$ BHIB were incubated for an additional 24 h at 25 °C. This resulted in approximately 10^8 planktonic *L. monocytogenes* cells/mL and 10^6 attached *L monocytogenes* cells per cm^2 inner surface.

Model drain pipes were approximately 7.7 times smaller than the maximum volume of the animal containment room drain pipes used in the uninoculated floor drain study. Therefore, for purposes of comparison, model drains were treated with custom-loaded ClO_2 pods with 7.5 times less precursor chemical (10 g of each precursor chemical). Empty pods and both proprietary chemical precursors (sodium chlorite and sodium bicarbonate based) were purchased separately [15]. Pods were placed in the open end of model drains using the same rubber adapter described for uninoculated floor drains. Two model drains were exposed to the ClO_2 from pods for 4 h, and 2 were exposed for 24 h. Two untreated control model drains were included for each time period. Five replications were conducted for a sample size of 10 treated and 10 control model drain pipes for each time of exposure.

In a second set of model drain replications, treatments and time of exposure were the same except pipes were sealed with lab film [22] in an effort to maximize ClO_2 containment within the pipe. Sealing was accomplished by a double layer of lab film connecting the pod to the rubber adapter and another double layer connecting the adapter to the model drain pipe. As with unsealed work, 5 replications of sealed pipe exposure were conducted with 2 pipes per treatment and exposure time.

Following 4 or 24 h of ClO_2 treatment, treated and control model drain pipes were sampled to determine the numbers of planktonic *L. monocytogenes* cells remaining viable in the liquid and the number of viable attached cells on the inner surface. For planktonic cell count, liquid was removed directly from the model drain by pipet; serial dilutions were prepared and plated as described below. For attached cells, model drains were emptied, rinsed three times with sterile water to remove unattached cells and the inner surface sampled by sponge wipe using a 4-cm ×4-cm sterile sponge [23] previously moistened with 5 mL of DE neutralizing broth [24] to inactivate lingering chlorine residue. Sponges were used to wipe around the bottom 4 cm of the entire inner pipe circumference for 3 consecutive passes.

Bacterial Enumeration

Nine milliliters of PBS was added to each sponge sample within its sterile bag. All sponge samples were subjected to 30 s of mixing in a paddle mixer [25]. Serial dilutions were prepared in PBS and plated onto the surface of LSA. Liquid samples for planktonic cell counts were serially diluted in PBS and plated onto the surface of LSA. All LSA plates were incubated at 35 °C for 24 h. Colonies characteristic of *L. monocytogenes* were counted and recorded as cfu/sponge sample or cfu/mL drain liquid.

Statistical Analysis

Colony-forming unit (**cfu**) counts were log-transformed and means were calculated. For naturally contaminated, uninoculated floor drains, mean log cfu/sponge attached cells from before and after treatment were compared using Student's *t* test. Significance was assigned at $P < 0.01$. For *L. monocytogenes* inoculated model drain pipes, mean log cfu/mL drain liquid for each treatment and time were compared by general linear model. Means were further separated by Tukey's honest significance test and significance was assigned at $P < 0.05$. Statistical analysis was conducted using a statistical analysis program [26].

RESULTS

Results from the ClO_2 treatment of naturally-occurring drain microflora in existing floor drains are presented in Table 1. Total aerobic bacteria detected before treatment was more than 10^6 cfu per sponge. After treatment the mean number detected from the other half of the pipe was less than 10 cfu. This represents approximately a 6 log decrease, more than 99.999% less total aerobic bacteria recovered after treatment. Control pipes (1 per replication for a total of 2)

Table 1. Mean log cfu total aerobic bacteria recovered per sponge from the inner surface of floor drain pipes before and after 4 h treatment with ClO_2 (n = 10).

Sample timing	Total aerobic cfu/sponge
Before treatment (back half of pipe)	6.6 ± 0.3^{A}
After treatment (front half of pipe)	0.5 ± 0.5^{B}

^{AB}values with different superscripts are different by Student's *t* test ($P < 0.01$).

were left untreated, while treated pipes were exposed to ClO_2. The front and back half of control drain pipes were examined before and after the 4 h non-treatment time and experienced less than one log decrease (from log 6.5 to log 5.7 cfu per sponge).

Results of ClO_2 treatment of lab scale model drains are presented in Tables 2 and 3. Table 2, shows data from five replications conducted without lab film to seal the pod to the pipe. A 4-h ClO_2 treatment significantly lowered the numbers of both planktonic and attached *L. monocytogenes* in the model floor drains. A 24-h treatment was even more effective, resulting in an almost 8 log cfu/mL decrease in free-swimming cells and more than 4 log cfu decrease in attached cells. Data from model drain pipes to which ClO_2- generating pods were sealed with lab film are presented in Table 3. Here we found that 4-h ClO_2 treatment eliminated viable *L. monocytogenes* in the drain liquid and lead to a more than 4 log decrease in attached cells. After 24 h, more than a 6 log (> 99.9999%) decrease in attached *L. monocytogenes* was observed.

DISCUSSION

Herein we report that ClO_2 treatment of a drain can decrease planktonic *L. monocytogenes* numbers by more than 8 log cfu/mL and attached *L. monocytogenes* by approximately 6 log cfu. An earlier report describes ClO_2 causing almost total elimination of *L. monocytogenes* attached to stainless steel [13]. In the current work, we were treating PVC instead of stainless steel, which may explain a more moderate decrease in numbers. Foschino et al. [14] reported that ClO_2 treatment was substantially less effective against *Escherichia coli* attached to PVC as opposed to stainless steel. They explained the difference by electron microscopic observation of the PVC surface, which was rough compared to stainless steel and included pores larger than bacterial cells [14].

Table 2. Mean log cfu/mL planktonic and attached (per sponge) *L. monocytogenes* recovered from inoculated model drain pipes treated by ClO_2 pod for 4 or 24 h (n = 10).

	Planktonic		Attached	
Treatment	4 h	24 h	4 h	24 h
Control	8.8 ± 0.1^{A}	8.7 ± 0.1^{A}	8.8 ± 0.2^{A}	8.7 ± 0.1^{A}
ClO_2	3.2 ± 2.3^{B}	0.9 ± 0.9^{C}	6.6 ± 0.9^{B}	4.0 ± 2.1^{C}

^{ABC}values within sample type (planktonic or attached) with different superscripts are different by Tukey's Honest Significant Difference Test ($P < 0.05$).

Table 3. Mean log cfu/mL planktonic and attached (per sponge) *L. monocytogenes* recovered from inoculated model drain pipes sealed with flexible lab film treated by ClO_2 pod for 4 or 24 h (n = 10).

	Planktonic		Attached	
Treatment	4 h	24 h	4 h	24 h
Control	8.7 ± 0.1^{A}	8.6 ± 0.1^{A}	8.5 ± 0.2^{A}	8.6 ± 0.1^{A}
ClO_2	nd^{B1}	nd^{B}	4.3 ± 1.5^{B}	1.9 ± 1.5^{C}

^{ABC}values within sample type (planktonic or attached) with different superscripts are different by Tukey's Honest Significant Difference Test ($P < 0.05$).

[1]none detected; theoretical limit of detection, 1 cfu/mL, used for statistical analysis.

Other chemicals and treatments have been tested for killing *L. monocytogenes* attached to different types of surfaces with varying degrees of success [27, 28, 29, 30, 31]. Relatively few studies have been conducted in model PVC drain pipes [32, 33]. In an earlier study, we reported that a peroxide-based chemical was more effective to lessen the numbers of *L. monocytogenes* attached to the inner surface of PVC model floor drains than either a chlorine- or quaternary ammonium-based chemicals [32]. Moist heat or steam has been examined as a means to kill bacteria, including *L. monocytogenes* attached to stainless steel [27, 31]. We have also reported that heating drain water to 95 °C for 15 s results in a 7 log cfu decrease in attached *L. monocytogenes* attached to the inner surface of PVC model drain pipes [33].

The self-contained ClO_2 generation and delivery system described in the current study provides a decrease in planktonic and attached *L. monocytogenes* cells that is comparable to other means of drain decontamination previously reported. The practical and convenient nature of the ClO_2 pod makes it a reasonable choice for periodic decontamination of floor drains. By generating only a small volume of ClO_2 gas and deploying it directly into the drain, risk of gas leakage can be managed, thereby minimizing risk of worker exposure. This technology would be best applied during periods of plant non-use so that the pods can be left in the drain for 24 hours, allowing for complete treatment and gas dissipation prior to worker re-entry and drain use.

CONCLUSIONS AND APPLICATIONS

1. Self-contained ClO_2 generating and delivery pods are commercially available and are effective to significantly lower numbers of inoculated *L. monocytogenes* in model PVC floor drains by up to 99.999999%.
2. Numbers of both planktonic (free-swimming) and to a somewhat lesser degree surface-attached *L. monocytogenes* are affected by the ClO_2 treatment tested in this study.
3. Natural bacterial populations in actual floor drains are similarly impacted by treatment with ClO_2 pods.
4. Self-contained ClO_2 pods have utility for periodic decontamination of floor drains in poultry processing and further processing plants thereby lessening the potential for *L. monocytogenes* escape and contamination of product or product contact surfaces.

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