

In cooperation with The Ohio State University

Results of an Evaluation of the Effectiveness of Chlorine Dioxide as a Disinfectant for Onsite Household Sewage Treatment Systems

Open-File Report 2011–1096

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By Christopher M. Kephart and Donald M. Stoeckel

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Conversion Factors

Multiply	By	To obtain
gallon (gal)	3.785	liter (L)
liter (L)	0.264	gallon (gal)

Concentrations of chemical constituents in water are given in milligrams per liter (mg/L).

Results of an Evaluation of the Effectiveness of Chlorine Dioxide as a Disinfectant for Onsite Household Sewage Treatment Systems

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Abstract

Scientists with the U. S. Geological Survey and The Ohio State University evaluated the effectiveness of chlorine dioxide (ClO_2) as a wastewater disinfectant for onsite household sewage treatment systems. Effectiveness of ClO_2 was measured by evaluating the removal of six test microorganisms: *Escherichia coli*, enterococci, *Clostridium perfringens*, *Bacillus anthracis* Sterne strain spores, somatic coliphage, and F-specific coliphage. These evaluations were done in bench-scale experiments and in a field-scale experiment where ClO_2 was delivered to the sewage by use of a novel dry packet system. Results presented herein demonstrate effective inactivation of each test microorganism except for *B. anthracis* spores.

Introduction

As communities in Ohio continue to work toward eliminating pollutant discharge to streams to meet goals of the Clean Water Act, wastewater reuse through onsite wastewater treatment followed by irrigation is an important option. For example, it has been estimated that only 6.4 percent of Ohio soils are suitable for traditional leach-line or mound septic systems (Mancl and Slater, 2002). Consequently, on the basis of calculations using distribution of soil types, an estimated 27 percent of all existing onsite household sewage treatment systems (HSTS) in Ohio are failing to adequately treat sewage effluent (Mancl, 1990). A solution to this problem may be to collect and disinfect HSTS effluent to make it safer for discharge into the environment and possible reuse as irrigation water.

Recycling or reuse of wastewater is a process that utilizes treated sewage in a manner that minimizes or eliminates the discharge of pollutants to resource waters. Reuse of wastewater, however, must be done with consideration of public health risk. Waterborne diseases, ranging from gastroenteritis to hepatitis, are caused by viruses, bacteria, and parasites that are spread through the fecal waste of infected persons. Wastewater from septic systems must be effectively disinfected to

minimize the risk of pathogen breakthrough to nearby streams and ditches and subsequently minimize the incidence of waterborne disease in the reuse setting.

Chlorine in multiple forms has been used as a disinfectant for wastewater. After its introduction in the 1950s, chlorine dioxide has recently reemerged as a disinfection agent. Chlorine dioxide has different activity than that of free chlorine or hypochlorite. Unlike free chlorine and hypochlorite, chlorine dioxide is active over a wide range of pH, and its use does not result in the creation of disinfection byproducts (Lykins and Griese, 1986). One disinfection byproduct that forms from the reaction of free chlorine with organics is trihalomethane, a halogenated hydrocarbon that has been linked to increased cancer mortality rates (Alavanja and others, 1980). Some studies have shown that microbial disinfection with chlorine dioxide is as effective or superior to disinfection with free chlorine—particularly in regard to inactivation of viruses (Narkis and Kott, 1992; Junli and others, 1997). Chlorine dioxide traditionally has been generated by combining sodium chlorite with free chlorine gas. Until now, the expense associated with the handling and injection of chlorine gas has made use of chlorine dioxide economical only for large water and wastewater facilities. A dry-packet delivery system (Avantec Technologies Inc., Columbus, Ohio) has recently made the chemistry behind chlorine dioxide generation safer and more economical, enhancing its usefulness for small treatment systems.

Widespread use of a newly developed chlorine dioxide disinfection system would depend, in part, upon its ability to inactivate pathogenic microorganisms in sewage effluent to make the water safe for reuse. The inactivation capability of chlorine dioxide can be described by the product between the chlorine concentration (C) and contact time (t) required for a specific percentage of microorganism removal and is expressed as a chlorine contact time (Ct) (Gaudy and Gaudy, 1980). In one study, the disinfection capabilities of free chlorine and chlorine dioxide in activated municipal sludge were compared. For approximately 7 mg/L chlorine dioxide, a 5-log decrease in fecal coliform concentration was achieved after 20 minutes of contact time ($Ct = 140 \text{ mg}\cdot\text{min/L}$). The same chlorine dioxide concentration caused a 6-log decrease

in coliphage concentration after 20 minutes of contact time. In contrast, the same Ct value for free chlorine resulted in a 5-log decrease in fecal coliform concentration, but only a 1-log decrease in coliphage concentration (Narkis and Kott, 1992).

The U.S. Geological Survey (USGS), in cooperation with The Ohio State University, evaluated the effectiveness of chlorine dioxide as a disinfection tool for household sewage treatment systems. The overall purpose of this study was to measure the ability of a novel chlorine dioxide delivery system to inactivate pathogens in onsite residential wastewater and thereby render the treated wastewater more suitable for discharge into the environment or reuse as irrigation water.

One specific objective of this study was to characterize bench-scale Ct requirements to describe the inactivation kinetics of chlorine dioxide for different types of microorganisms in a wastewater matrix (fecal-indicator bacteria, spore-forming bacteria, and viruses). Another objective was to determine the effectiveness of the chlorine dioxide system against the same suite of microorganisms in a field-scale operational setting—an onsite, single-family household sewage treatment system. Methods and results from these two experimental approaches are described herein.

Methods

The effectiveness of chlorine dioxide was tested in wastewater collected from the Molly Caren Agricultural Center wastewater stabilization pond, part of a three-person household septic system established and maintained as an educational tool by The Ohio State University.

Selection of Target Microorganisms

Six different microorganisms were used to evaluate chlorine dioxide effectiveness: *Escherichia coli*, enterococci, *Clostridium perfringens*, *Bacillus anthracis* spores (Sterne), F-specific coliphage, and somatic coliphage. *E. coli* and enterococci were selected for study because they are the required indicators for monitoring and regulation of recreational waters for fecal contamination (U.S. Environmental Protection Agency, 1986, 2004). *C. perfringens* is an alternate indicator of fecal contamination (Hill and others, 1996). *C. perfringens* is found both as vegetative cells and endospores. *B. anthracis* Sterne is a nontoxigenic strain of a spore-forming zoonotic pathogen. Evaluation of chlorine dioxide in reducing spore formers would provide a measure of effectiveness against stress-resistant microorganisms. F-specific and somatic coliphages are viruses that infect *E. coli* by two different mechanisms. F-specific coliphages are RNA or DNA viruses that infect via the F-pilus of male strains of *E. coli*. Somatic coliphages are DNA viruses that infect via the outer cell membrane. These coliphage have been used as surrogate organisms to indicate capacity for transport and survival of enteric viruses from

fecal contamination in water (Skraber and others, 2004; Helmi and others, 2008).

Wild type enrichments of each of the test microorganisms except for *B. anthracis* were cultivated from raw sewage from the Olentangy Environmental Control Center, a wastewater treatment plant in Delaware County, Ohio. Stocks of the test microbes were selected for and enriched immediately before each experimental trial. Each test microorganism was enumerated to determine concentrations after experimental treatment and, in some cases, to determine expected (theoretical) concentrations for seeding inocula. Specifically, *E. coli* was quantified by use of the modified mTEC membrane-filtration method 1603 of the U.S. Environmental Protection agency (USEPA) (2006b), enterococci was quantified by use of the mEI membrane-filtration method 1600 of the USEPA (2006a), *C. perfringens* was quantified by use of the mCP membrane-filtration method (Bisson and Cabelli, 1979), *B. anthracis* was quantified by spread plating onto PLET agar (Knisely, 1966), and F-specific and somatic coliphage were quantified by the single agar layer method 1602 of the USEPA (2001). Theoretical concentrations of each seed inoculum were determined by factoring in the starting volume of the experimental matrix. General laboratory quality-assurance procedures followed those of Francy and others (2010).

Bench-Scale Experiments

For each of the three bench-scale trials, two 10-L samples of wastewater collected near the outlet of the Molly Caren wastewater stabilization pond were used as the test matrix. One 10-L sample was used as a recovery control, and ClO_2 was not added to this sample carboy. The other 10-L volume was used as the test sample, and ClO_2 was added to this carboy. The wastewater was collected either the day of the trial or the day before and stored at room temperature.

Evaluation by means of bench-scale experiments was done in three trials. Wastewater was seeded with inocula of each of the test microorganisms listed above. Initial testing did not include *B. anthracis* spores; this organism was included in the evaluation after determining that the *C. perfringens* inocula consisted of predominantly vegetative cells and would not have represented a typical stress resistant spore-forming microorganism. As a result of the change, the bench-scale evaluation for the *B. anthracis* spores was only done once, and the field-scale evaluation did not include *C. perfringens* as a test microorganism. Each test microorganism was added to both carboys, and the seeded samples were mixed vigorously by shaking. Both carboys were then set up to stir slowly throughout the trial to keep an even distribution of the microorganisms but without creating significant aeration of the sample that might lower the effective ClO_2 concentration.

Sample collection began with a 1-L sample from the test carboy as the “time = 0” sample for microorganism analyses. For the bench-scale experiments, ClO_2 was pipette-transferred to the test carboy from a recently prepared ClO_2 solution and

allowed to mix for 30 seconds, then a 100-mL sample was collected as the initial sample for measuring ClO_2 concentration. Samples were subsequently collected (100 mL for ClO_2 concentration and 1 L for microorganism analyses) after 5, 10, 30, 60, and 120 minutes. Samples were also collected from the recovery control vessel at these time points. For the third trial test carboy, a sample was also collected at 2.67 minutes, and the 120-minute sample was eliminated from the series. For the third trial recovery control carboy, samples were collected only at 0 and 120 minutes. Each sample for microbial analyses was collected in a bottle containing sodium thiosulfate to neutralize any residual ClO_2 and was stored on ice immediately after collection. Samples were brought back to the USGS for analysis on the day of the trial.

Field-Scale Trial Procedures

Field-scale experiments were run in the final holding tank of the single-family household sewage treatment system at the Molly Caren site. Introduction of test microorganisms and ClO_2 to the approximately 1,000-gallon holding tank was done by first mixing in a 100-gallon auxiliary polypropylene tank that received a constant flow of the wastewater moving from the underground holding tank to the auxiliary tank. The wastewater in the auxiliary tank was returned to the holding tank via an overflow outlet near the midpoint of the auxiliary tank. Distribution of wastewater in the approximately half-full holding tank was done by use of two pumps: a low-flow sump pump added to the tank near the inlet of the auxiliary tank and a supplemental, high-flow pump added to the tank near the outlet at the far end of the holding tank.

Before the field-scale trial was run, a distribution experiment was done to determine whether, through settling or inadequate mixing, concentrations of microbes sampled at different locations in the tank would vary significantly. Additionally, this experiment would demonstrate whether or not significant die-off would occur over time in the final holding tank. An inoculum of *E. coli*, prepared in the same manner as in the bench-scale experiments, was added to the auxiliary tank and was mixed by use of both pumps for 30 minutes. The initial samples were then collected at five different locations in the holding tank by using a peristaltic pump and sterile tubing. The high-flow, supplemental pump was then shut off. Single samples were then collected at the southwest corner of the holding tank after 60, 90, 150, and 165 minutes. A concurrent replicate sample was collected for the 60-minute sample. The location of these samples was the furthest from the mixing of the low-flow sump pump and was thought to represent a worst-case scenario for observing settling or inadequate mixing. Another step taken to determine whether mixing was not adequate was that after the 150-minute sample was collected, the high-flow, supplemental pump was turned back on for 15 minutes before collection of the 165-minute sample.

One field-scale paired trial was done on two separate days to evaluate ClO_2 effectiveness; that is, recovery control samples (not treated with ClO_2) were collected and analyzed

one day and test samples (treated with ClO_2) were collected and analyzed another day. For this paired trial, the experimental setup was the same as described above for the distribution experiment except that ClO_2 was added to the wastewater in the auxiliary tank by use of the Avantec-prepared dry packets, and the sampling time intervals were not the same. Five test microbes (*E. coli*, enterococci, *B. anthracis*, F-specific coliphage, and somatic coliphage) were introduced to the auxiliary tank, and high-flow mixing was done with both pumps for 30 minutes. All samples were collected in the same manner as in the distribution experiment. The time = 0 sample was collected and the high-flow pump was then shut off. For the recovery control experiment, subsequent samples were collected after 5, 30, and 120 minutes. For the ClO_2 test experiment, the disinfectant was added after the 30-minute high-flow mixing, and subsequent samples were collected after 5, 30, 90, 180 and 190 minutes. After the 180-minute test sample, the high-flow, supplemental pump was turned back on for 10 minutes before collection of the 190-minute sample. Concurrent replicate samples were collected after the 30-minute sample for both the recovery control and test samples. All samples for the field-scale experiments were collected in sterile 1-L bottles amended with sodium thiosulfate and were preserved on ice immediately following collection for onsite processing within 1 hour.

Results

Bench-Scale ClO_2 Inactivation Capabilities

Chlorine dioxide effectiveness was measured for six different microorganisms seeded into wastewater collected from the Molly Caren pond-based septic system. Concentrations of each test microorganism for each of three trials are shown in table 1. For *B. anthracis*, only one bench-scale trial was done. For the test carboys, concentrations at time = 0 indicate initial microorganism levels after initial mixing in the wastewater but before the introduction of ClO_2 . For the test microcosm in trial 3, a sample-collection time 2 minutes 40 seconds (2.67 minutes) following the addition of ClO_2 was added to help better describe microbe die-off characteristics in the early stages of ClO_2 exposure. For the recovery control carboys, sample-collection time steps were reduced to two for trial 3 and three for the *B. anthracis* trial because steady concentrations were observed during trials 1 and 2.

Rapid, high levels of die-off were observed in each of the bench-scale trial test carboys for each microorganism tested except for *B. anthracis*. After 5 minutes of ClO_2 exposure, the $\log_{10}$ -removal ranged from 4.22 to 5.97 CFU/100 mL for *E. coli*, 4.06 to 6.57 CFU/100 mL for enterococci, 1.70 to 5.08 CFU/100 mL (initial concentration in trial 2 was only 2.70 log CFU/100 mL) for *C. perfringens*, 4.60 to 5.68 CFU/100 mL for somatic coliphage, and 5.87 to 7.15 for F-specific coliphage. Factoring in the time of ClO_2 exposure allows for the calculation of *Ct* values for these data. While

Table 1. Test microorganism concentrations following three bench-scale trials of chlorine dioxide effectiveness for disinfection of wastewater. Effectiveness against *B. anthracis* was evaluated in only one trial.

[All data are concentrations, in colony-forming units per 100 milliliters for bacteria and plaque-forming units per 100 milliliters for coliphage; —, sample not collected; <, no positive results for lowest volume evaluated]

Test samples (treated with ClO ₂)														
Time (minutes)	E. coli			Enterococcus			C. perfringens			Bacillus anthracis	Coliphage			
	Trial 1	Trial 2	Trial 3	Trial 1	Trial 2	Trial 3	Trial 1	Trial 2	Trial 3		Somatic			F-specific
											Trial 1	Trial 2	Trial 3	
0	2.8E+06	5.1E+07	3.5E+07	5.6E+06	6.7E+06	4.7E+07	1.2E+06	5.0E+02	4.2E+06	3.4E+06	2.4E+05	1.5E+06	7.5E+05	1.7E+08 7.3E+07 5.4E+08
2.67	—	—	400	—	—	1200	—	—	110	—	—	—	7	— 280
5	3	310	300	<3	580	700	10	10	33	4.7E+06	<1	38	3	12 99 110
10	3	140	180	10	220	430	3	5	30	3.3E+06	<1	22	7	28 200 78
30	3	150	210	<3	190	520	<3	<3	13	4.7E+06	<1	26	2	100 160 94
60	<3	170	190	<3	140	590	<3	<3	10	2.9E+06	<1	57	1	3 110 73
120	<3	150	—	<3	140	—	<3	<3	—	2.6E+06	<1	35	—	4 50 —

Recovery control samples (no ClO ₂)														
Time (minutes)	E. coli			Enterococcus			C. perfringens			Bacillus anthracis	Coliphage			
	Trial 1	Trial 2	Trial 3	Trial 1	Trial 2	Trial 3	Trial 1	Trial 2	Trial 3		Somatic			F-specific
											Trial 1	Trial 2	Trial 3	
0	3.2E+06	3.2E+06	2.6E+07	6.5E+06	5.4E+06	3.5E+07	2.8E+05	9.0E+02	5.5E+06	4.2E+06	2.6E+05	2.0E+06	6.0E+05	1.2E+08 4.5E+07 6.1E+08
5	4.4E+06	4.8E+06	—	5.8E+06	5.9E+06	—	1.5E+06	5.0E+02	—	—	2.5E+05	2.3E+06	—	1.1E+08 4.7E+07 —
10	2.2E+06	2.8E+06	—	6.0E+06	3.8E+06	—	<1.0E+05	3.3E+02	—	—	2.8E+05	1.8E+06	—	8.8E+07 5.0E+07 —
30	3.1E+06	4.1E+06	—	5.6E+06	5.1E+06	—	8.6E+05	3.1E+02	—	3.6E+06	2.4E+05	2.0E+06	—	9.3E+07 4.2E+07 —
60	2.9E+06	5.5E+06	3.6E+07	5.5E+06	6.1E+06	3.5E+07	2.3E+05	1.5E+02	4.0E+05	—	1.9E+05	1.8E+06	4.8E+05	1.1E+08 4.3E+07 5.4E+08
120	3.3E+06	3.1E+06	—	5.2E+06	5.2E+06	—	8.4E+05	1.2E+03	—	3.0E+06	2.3E+05	1.8E+06	—	1.1E+08 2.7E+07 —

ClO_2 concentrations are included as a function of the Ct values, the raw data for these concentrations are not presented. Plots of Ct values versus $\log_{10}$ -removal in CFU/100 mL are presented in figure 1.

Field-Scale ClO_2 Inactivation Capabilities

Chlorine dioxide effectiveness was also measured in a 1,000-gallon septic holding tank. Before challenging ClO_2 against the test microorganisms, an initial experiment was run to determine how well distributed the microorganisms were in the large tank. Results from this experiment are presented in figure 2. *E. coli* concentrations for the initial five samples collected at various locations in the tank after 30 minutes of high-flow agitation ranged from 6.26 to 6.45 $\log_{10}$ CFU/100 mL, thus demonstrating reasonably even distribution throughout the tank. These concentrations corroborated theoretical concentrations based on an estimate of the seed inoculum concentration of 6.52 $\log_{10}$ CFU/100 mL. The subsequent samples collected (under low-flow agitation) further supported that the *E. coli* were distributed evenly throughout the tank and also demonstrated that significant native die-off was not

occurring during the allotted time. Additionally, the final sample collected after vigorous agitation reinforced that seeded *E. coli* did not settle out significantly in the tank throughout the 165-minute experiment.

The paired trial results for the field-scale ClO_2 evaluation are presented in table 2 and figure 3. Overall, removal caused by ClO_2 exposure in the field-scale trial was not as rapid or severe as in the bench-scale trials. A delay in the initial effect of the disinfectant was expected, given that the ClO_2 was delivered to the system in dry-packet form that required more time to activate and, once in solution, the ClO_2 required time of travel from the auxiliary tank to the large holding tank where the samples were collected. For the test samples, $\log_{10}$ -removal peaked for all microorganisms except *B. anthracis* after 180 minutes of disinfectant exposure. Somewhat contrary to the field-scale distribution experiment, some decreases were seen in microorganism concentrations after high-flow agitation was reinitiated ($time = 190$ minutes), indicating that some settling had occurred during the experiment. Corroborating the results from the bench-scale experiments, ClO_2 exposure was ineffective against the *B. anthracis* spores in the conditions established for the field-scale experiment.

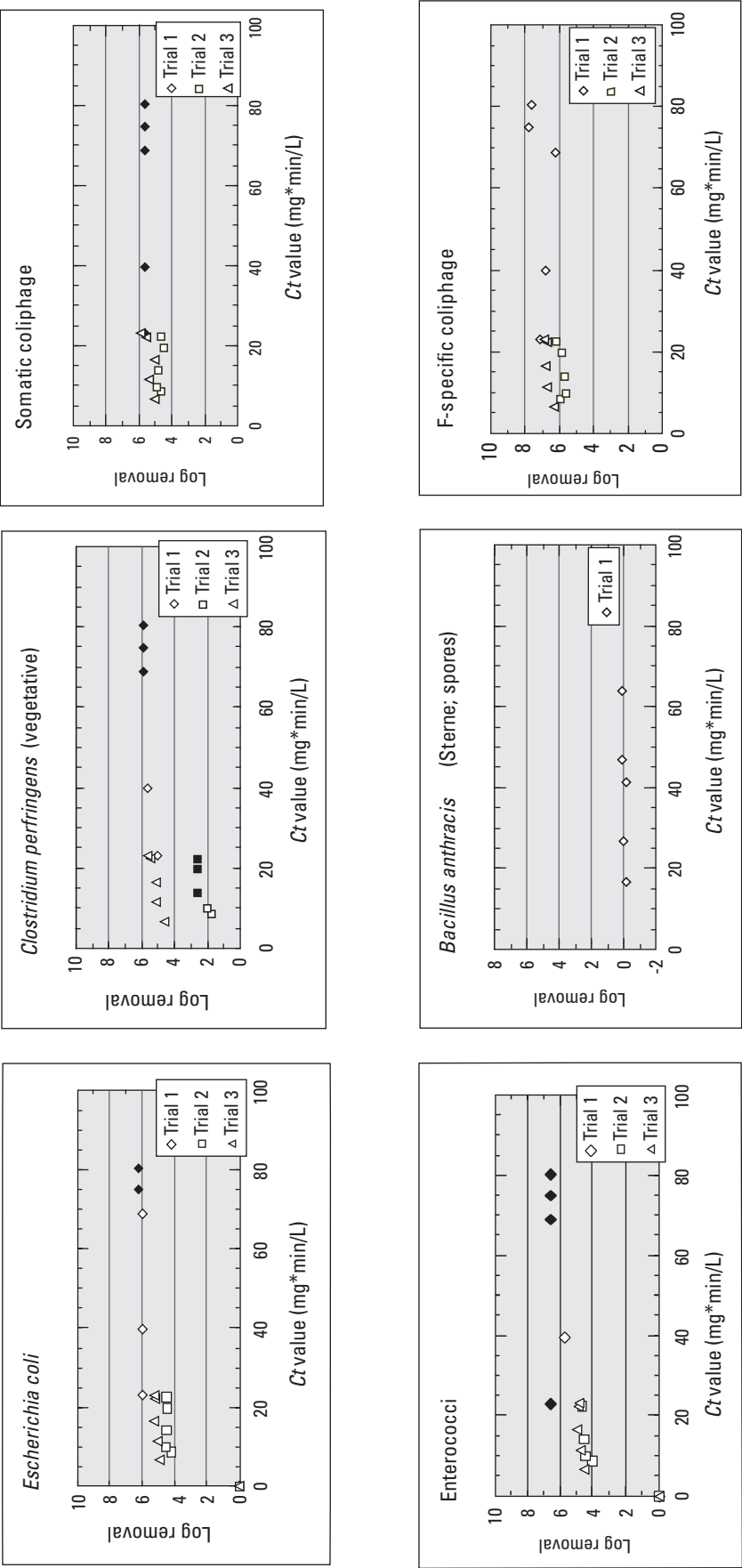


Figure 1. Results of bench-scale dose-response of target microorganisms in experiments with chlorine dioxide as a wastewater disinfectant. Shaded data points indicate none detected; removal indicated is a minimum value.

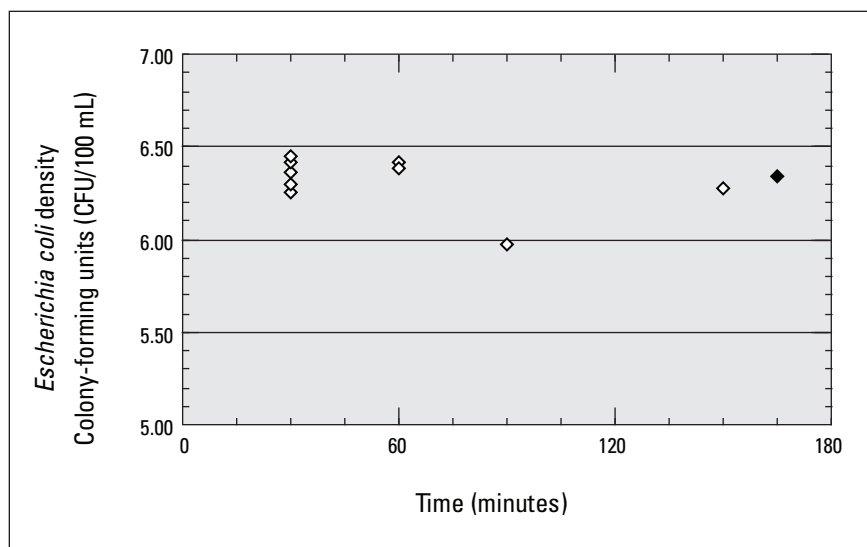


Figure 2. Distribution and persistence of *Escherichia coli* in the holding tank at the Molly Caren Agricultural Center site, in the absence of chlorine dioxide disinfectant. Samples were collected at five locations in the tank at the first sample time (after 30 minutes of mixing). A concurrent replicate sample was collected for the 60-minute sample. Shaded data point indicates density measured following vigorous agitation (the difference between the density at 150 minutes and 165 minutes is attributable to settling).

Table 2. Test microorganism concentrations following a field-scale trial of chlorine dioxide effectiveness for disinfection of wastewater.

[CFU/100 mL, colony-forming units per 100 milliliters; PFU/100 mL, plaque-forming units per 100 milliliters]

Test samples (treated with ClO ₂)										
Coliphage										
	<i>E. coli</i>		Enterococcus		<i>B. anthracis</i>		Somatic		F-specific	
Time (minutes) ¹	CFU/100 mL	Log remaining	CFU/100 mL	Log remaining	CFU/100 mL	Log remaining	PFU/100 mL	Log remaining	PFU/100 mL	Log remaining
0	2.7E+06	6.4	1.5E+06	6.2	1.9E+06	6.3	1.9E+06	6.3	2.2E+07	7.3
5	2.6E+06	6.4	1.9E+06	6.3	1.5E+06	6.2	2.2E+05	5.3	2.1E+07	7.3
30	8.7E+05	5.9	9.3E+05	6.0	1.0E+06	6.0	4.5E+04	4.7	1.0E+06	6.0
31 ²	8.9E+05	5.9	6.8E+05	5.8	1.3E+06	6.1	3.0E+04	4.5	9.0E+05	6.0
90	3.5E+03	3.5	1.4E+04	4.1	2.0E+06	6.3	1.8E+04	4.3	1.3E+04	4.1
180	73	1.9	1.2E+03	3.1	1.8E+06	6.3	1.3E+03	3.1	1.3E+03	3.1
190 ³	1.5E+03	3.2	4.6E+03	3.7	2.1E+06	6.3	4.2E+03	3.6	1.4E+03	3.1
Recovery control samples (no ClO ₂)										
Coliphage										
	<i>E. coli</i>		Enterococcus		<i>B. anthracis</i>		Somatic		F-specific	
Time (minutes)	CFU/100 mL	Log remaining	CFU/100 mL	Log remaining	CFU/100 mL	Log remaining	PFU/100 mL	Log remaining	PFU/100 mL	Log remaining
0	3.4E+06	6.5	1.9E+06	6.3	1.4E+06	6.1	1.2E+06	6.1	2.4E+07	7.4
5	3.2E+06	6.5	1.5E+06	6.2	1.7E+06	6.2	1.0E+06	6.0	2.1E+07	7.3
30	4.3E+06	6.6	1.9E+06	6.3	1.6E+06	6.2	1.4E+06	6.1	1.2E+07	7.1
31 ²	4.5E+06	6.7	1.8E+06	6.3	1.6E+06	6.2	1.6E+06	6.2	1.9E+07	7.3
120	3.8E+06	6.6	2.3E+06	6.4	2.0E+06	6.3	1.3E+06	6.1	3.4E+07	7.5

¹All microorganisms inoculated, mixed with high-flow agitation for 30 minutes, time = 0 collected, then supplemental pump deactivated.

²Concurrent replicate sample collected immediately following 30-minute sample.

³Supplemental pump reactivated following collection of 180-minute sample, run for 10 minutes, then final sample collected (time = 190).

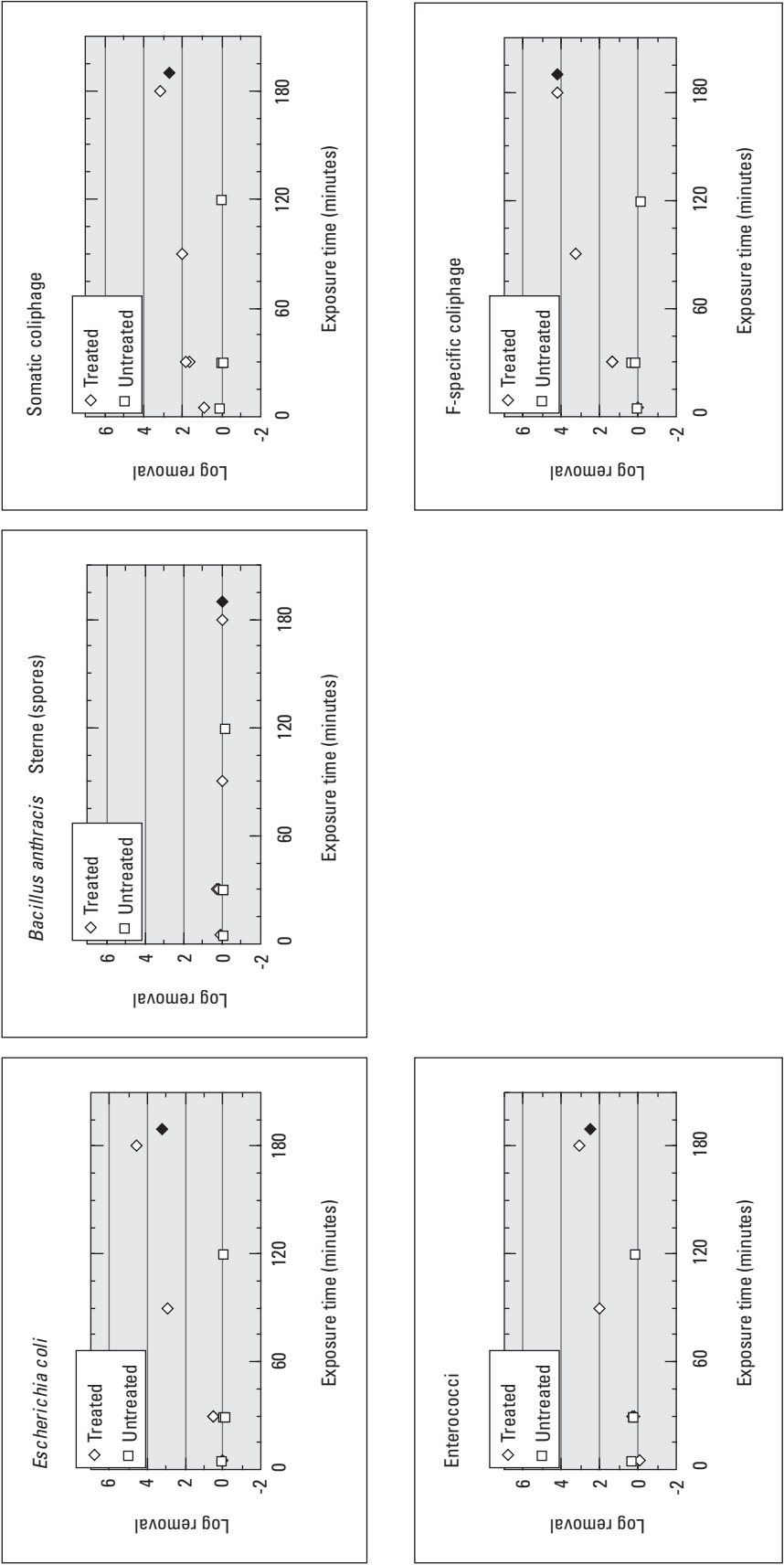


Figure 3. Results of field-scale trial of chlorine dioxide disinfection packets for target microorganisms at a single-family household sewage treatment system (HSTS). Shaded data points indicate densities measured following vigorous agitation (the difference between the density at 180 minutes and 190 minutes is attributable to settling).

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