Technology Notes
Bacterial Efficacy Testing

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Introduction

The antimicrobial properties of ozone are well documented. Ozone has been used in the municipal water treatment industry for decades. Ozone is recognized and validated as a hard surface sanitizer in the food processing industry, including meat, poultry, and fish processing facilities. Ozonated water, or Aqueous Ozone, is now emerging as an effective cleaner that may be utilized in a variety of cleaning environments. It can be used on a variety of surfaces from floors and drains to walls, tanks, and even soft surfaces such as carpet. The challenge for Aqueous Ozone, and companies such as CleanCore, is the maze of environmental regulations and testing required to make product claims as a sanitizer.

CleanCore Technologies has harnessed the power of ozone in a solution that is generated on-site and on-demand thereby offering products that produce a truly non-synthetic, green cleaning solution. In an effort to provide transparency to its customers, CleanCore has engaged the University of Nebraska Medical Center (UNMC) to conduct independent laboratory studies to document the bacterial kill efficacy or antimicrobial properties of the aqueous ozone solution created by its products at varying concentrations. The following is a summary of testing conducted and results obtained.

Testing Overview

UNMC was engaged to conduct multiple bacterial kill efficacy tests as follows:

1. Initial, Phase One testing was completed using university lab designed tests, not standard testing, against *Escherichia coli and Listeria monocytogenes.* Multiple surfaces were contaminated and then treated with two concentrations of aqueous ozone, 1.5 ppm and 3.5 ppm, respectively. Two contact or dwell times, 2 minutes and 30 minutes, were tested to evaluate the bacterial kill efficacy of the CleanCore aqueous ozone solution.

2. Phase two testing was completed using the standard test method as prescribed in EPA Guidelines contained in *EPA OCSPP 810.2300 "Product Performance Test Guidelines"*. More specifically, standard test method *ASTM 1153-14 "Efficacy of Sanitizers Recommended for Inanimate, Hard, Nonporous, Non-Food Contact Surfaces"* was utilized to test the following bacteria:
    a. Escherichia coli
    b. Listeria monocytogenes
    c. Salmonella enterica
    d. Enterobacter aerogenes
    e. Klebsiella pneumoniae
    f. Enterococcus faecalis.

Under the EPA prescribed test method, a reduction of 99.9% or greater in the number of test organisms over the control samples is required to "pass" the test and be considered a hard surface sanitizer. The dwell or contact time prescribed is 5 minutes.

3. Phase three testing was completed using the same testing methodology as Phase two except the dwell or contact time of the aqueous ozone used in the tests was reduced to one and two minutes. Specifically, additional tests were completed on Escherichia coli and Listeria monocytogenes. These tests were completed to help evaluate the shortest effective contact time for the CleanCore aqueous ozone solution.

Test Results Summary-

Phase One – Custom Designed Methodology by university

				% Reduction on Coupon		% Reduction in Supernatant (run-off)	
Bacteria	Exposure/ Contact Time	Ozone Concentration	Average CFU Load by Absorbance	Ceramic Tile	Stainless Steel	Ceramic Tile	Stainless Steel
E. coli	2 minutes	1.5 PPM	19,063,000	100%	100%	100%	100%
	30 minutes	1.5 PPM	19,063,000	100%	100%	100%	99.99%
Listeria	2 minutes	1.5 PPM	3,030,000	99.99%	100%	99.94%	99.66%
	30 minutes	1.5 PPM	3,030,000	99.98%	99.99%	99.87%	99.55%
E. coli	2 minutes	3.5 PPM	19,063,000	100%	100%	100%	100%
	30 minutes	3.5 PPM	19,063,000	100%	100%	100%	100%
Listeria	2 minutes	3.5 PPM	3,030,000	100%	100%	99.96%	99.98%
	30 minutes	3.5 PPM	3,030,000	100%	100%	99.95%	99.42%

"Overall, it appears, based on the studies executed that aqueous ozone was able to reduce the CFU on both stainless steel and ceramic surfaces from E coli contamination."

"With Gram Positive bacteria Listeria monocytogenes, aqueous ozone was able to significantly reduce the CFU found on the coupon surface on both stainless steel and ceramic coupons.

Phase Two – *ASTM 1153-14 Efficacy of Sanitizers Recommended for Inanimate, Hard, Nonporous, Non-Food Contact Surfaces*

Under this methodology there were 8 glass coupons coated with the bacterium solutions. Three of the coupons are control coupons treated with sterile water and 5 coupons are test coupons treated with 1.5 ppm aqueous ozone solution. The average CFU count from the control coupons is then compared to the average CFU count from the test coupons to determine a percentage reduction. The test methodology was completed in triplicate for each bacterium.

Results are summarized in the following table.

Bacteria	Ozone Concentration	Average Initial Plated CFU Load	Geometric Mean CFU count: Control Coupons	Geometric Mean CFU count: Test Coupons	% Reduction
E. coli	1.5 PPM	39,333,000	844,694	2	100.00%
Listeria	1.5 PPM	14,667,000	2,403,028	4,226	99.82%
Salmonella	1.5 PPM	3,450,000	280,663	165	99.94%
Enterobacter	1.5 PPM	18,916,000	3,401,819	21,689	99.36%
Klebsiella	1.5 PPM	11,500,000	2,093,656	31,995	98.47%
Enterococcus	1.5 PPM	87,067,000	1,410,713	77,451	94.51%

In each report issued for the individual bacterium, it was concluded that:

"After exposure to 1.5ppm of aqueous ozone for five minutes, there was a statistically significant decrease in bacteria CFU".

Phase Three - ***ASTM 1153-14 Efficacy of Sanitizers Recommended for Inanimate, Hard, Nonporous, Non-Food Contact Surfaces***

Utilizing the same methodology as Phase Two above, Phase three testing is designed to identify the shortest effective dwell or contact time for the CleanCore aqueous ozone solution. In this Phase, E. coli (Gram – bacteria) and Listeria (Gram + bacteria) were tested with dwell or contact times of one minute and two minutes as opposed to the five minutes in Phase two.

Results are summarized in the following table.

UNMC Phase Three Testing Summary Table

Bacteria	Exposure/ Contact Time	Ozone Concentration	Average CFU Load	Geometric Mean CFU count: Control Coupons	Geometric Mean CFU count: Test Coupons	% Reduction
E. coli	1 Minute	1.5 PPM	25,000,000	218,694	12	99.99%
	2 minutes	1.5 PPM	19,166,667	33,621	5	99.99%
Listeria	1 Minute	1.5 PPM	61,333,333	2,266,714	119,068	94.75%
	2 minutes	1.5 PPM	22,000,000	2,070,891	117,346	94.33%

E. coli report excerpt:

> *"the aqueous ozone was able to significantly decrease the number of live Escherichia coli on glass test squares with a 1-2 minute contact / dwell time with the E coli using this method"*

Listeria report excerpts:

> *"For the treatment conditions using freshly generated aqueous ozone for 1 or 2 minutes, there was a statistically significant decrease in Listeria CFU."*

NOTE: a scheduled preventative maintenance service on the testing unit had been delayed and had not been completed at the time of the Listeria tests. Subsequently, the PM service was completed and it was determined that a new air dryer was needed. Further, this means the system was not generating aqueous ozone at full efficiency and likely was generating closer to 1ppm of concentration as opposed to the 1.5ppm noted in the report. This likely explains the slightly lower Listeria test results noted in Objective 3 as compared to Objective 2.