

Published scientific studies conducted on behalf of Vollra by Dr. James Marsden at Kansas State University demonstrated that Vollara's ActivePure® technology substantially reduces microbial populations on surfaces. The study's results are being provided solely for informational purposes. The study's results have not been reviewed by the FDA, EPA or any other governmental agency. Our products are not medical devices and are not intended to diagnose, treat, or cure any disease.

Biological reductions on Surfaces

Efficacy of EcoQuest Radiant Catalytic Ionization Cell and Breeze AT Ozone Generators at Reducing Microbial Populations on Stainless Steel Surfaces

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Summary and Implications

This study was conducted to determine the potential use of EcoQuest Radiant Catalytic Ionization Cell for the inactivation of *Escherichia coli*, *Listeria monocytogenes*, *Streptococcus* spp., *Pseudomonas aeruginosa*, *Bacillus* spp., *Staphylococcus aureus*, *Candida albicans*, and *S. chartarum*, on stainless-steel surfaces at diverse contact times in a controlled airflow cabinet. In addition, the EcoQuest Breeze AT Ozone generator was evaluated under the same conditions for the inactivation of *Candida albicans* and *S. chartarum*. Better disinfection technologies for food contact surfaces are needed to control food borne pathogens in processing environments. Ozone technologies have only recently been approved for use on food contact surfaces. This study evaluated the application of gaseous ozone and other oxidative gases on stainless-steel surfaces against the microorganisms listed above. Both technologies reduced populations of all microorganisms tested on stainless-steel surfaces by at least 90% after 24 h exposure. The Radiant Catalytic Ionization Cell was more effective at reducing microbial counts for shorter exposure times than was the Breeze AT Ozone Generator.

INTRODUCTION

The food and beverage industries face a number of issues when it comes to producing a safe, wholesome product. Foodborne pathogens such as *E. coli* 0157:H7, *Listeria monocytogenes*, and *Salmonella* spp. have been a growing concern throughout the years. Processors are also concerned about spoilage microorganisms that shorten shelf life and cost companies millions every year in spoiled product. Industries impacted include the meat, seafood, poultry, produce, baking, canned foods, dairy, and almost all other segments of the market.

The U.S. Department of Agriculture estimates the costs associated with food borne illness to be about \$5.5 to \$22 billion a year. This doesn't include the billions lost every year due to spoiled product, which must be disposed of or sold as a lesser valued product. Better disinfection and microbiological control measures are needed in almost every area of the food industry.

As a disinfectant, ozone has a tremendous ability to oxidize substances. It's thousands of times faster than chlorine and disinfects water three to four times more effectively. As it oxidizes a

substance ozone will literally destroy the substance's molecule. It can oxidize organic substances such as bacteria and mildew, sterilize the air, and destroy odors and toxic fumes. Ozone has been used by industry for many years in numerous applications such as odor control, water purification, and as a disinfectant (Mork, 1993). Recent government approval of ozone for use with foods and food contact surfaces has opened the door to many more exciting possibilities for this technology.

In June 2001, the U.S. Food and Drug Administration approved the use of ozone as a sanitizer for food contact surfaces, as well as for direct application on food products. Prior to that time, chlorine was the most widely used sanitizer in the food industry. Ozone may be a better choice for disinfection of surfaces than chlorine. Chlorine is a halogen-based chemical that is corrosive to stainless steel and other metals used to make food-processing equipment. Chlorine can also be a significant health hazard to workers; when mixed with ammonia or acid cleaners, even in small amounts, a toxic gas can form.

Chlorine is a common disinfect used in meat processing and is effective and safe when used

at proper concentrations. However, chlorine is far less effective than ozone and can result in the production of chloroform, carbon tetrachloride, chloromethane, and tri-halomethanes. In contrast, ozone leaves no residual product upon its oxidative reaction.

An important advantage of using ozone in food processing is that the product can be called organic. An organic sanitizer must be registered as a food contact surface sanitizer with the U.S. Environmental Protection Agency (EPA). Ozone has such an EPA registration, and is approved by FDA as a sanitizer for food contact surfaces and for direct application on food products.

Ozone has become more accepted for use in food processing in recent years and is being used in more than just surface applications. A recent U.S. FDA recommendation (2004) stated that “ozone is a substance that can reduce levels of harmful microorganisms, including pathogenic *E. coli* strains and *Cryptosporidium*, in juice. Ozone is approved as a food additive that may be safely used as an antimicrobial agent in the treatment, storage, and processing of certain foods under the conditions of use prescribed in 21 CFR 173.368.”

MATERIALS AND METHODS

Preparation of Cultures:

The following bacteria and fungi cultures were used for the study: *Bacillus globigii* (ATCC # 31028, 49822, 49760), *Staphylococcus aureus* (ATCC # 10832D, 25178, 11987), *Candida albicans* (ATCC # 96108, 96114, 96351), *Stachybotrys chartarum* (ATCC # 18843, 26303, 9182), *Pseudomonas aeruginosa* (ATCC# 12121, 23315, 260), *Escherichia coli* (ATCC# 27214, 19110, 67053), *Streptococcus pneumoniae* (ATCC# 27945, 29514, 10782), and *Staphylococcus aureus* - methicillin resistant (ATCC# 33591). Cultures were revived using ATCC recommended instructions.

Bacteria, yeast, and mold strains were individually grown in tripticase soy broth (TSB; Difco Laboratories, Sparks, MD) and YM broth

(Difco Laboratories), respectively, to mid-exponential phase followed by a wash and re-suspension in 0.1% peptone water. The cultures were combined by specie type to ca. 10^8 CFU/ml.

Preparation of Samples and Ozone Treatment:

The microbial species used to validate the ozone generators were tested as microbial cocktails inoculated onto 6.3 x 1.8 cm on #8 finish stainless-steel coupons (17.64 cm² double sided area). Four stainless steel coupons were dipped per microbial inoculum and vortexed 15 sec to optimize microbial dispersion. Using sterile binder clips, stainless steel coupons were suspended on a cooling rack contained inside a laminar flow cabinet for 1 h to dry. The initial microbial populations attached to the stainless steel coupons ranged from 5 to 6 log CFU/cm². The inoculated stainless steel coupons were transferred to a controlled airflow test cabinet (Mini- Environmental Enclosure, Terra Universal, Anaheim, CA) at 26°C and 46% relative humidity (ambient conditions), and treated using the EcoQuest Radiant Catalytic Ionization Cell for 0, 2, 6, and 24 h. The EcoQuest Breeze AT Ozone generator was evaluated separately for treatment periods of 0, 2, 6 and 24 h. Ozone levels were monitored throughout the study (Model 500, Aeroqual, New Zealand).

Sampling:

At the end of the ozone contact time the coupons were vortexed for 30 sec in 30 ml of 0.1% peptone water. Samples inoculated with bacterial cultures were serially diluted, plated on tripticase soy agar (TSA; Difco Laboratories), and incubated for 24 h at 35°C. After preparing serial dilutions, samples inoculated with yeast were plated on potato dextrose agar (PDA; Difco Laboratories) and those inoculated with mold cultures were plated on cornmeal plates. Both PDA and cornmeal plates were incubated 30°C for 5 days. Following incubation, data for each microorganism were reported as colony-forming units per square centimeter (CFU/cm²).

RESULTS AND DISCUSSION

Reductions in microbial populations on #8 finish stainless steel coupons following 0, 2, 6, and 24 h exposure to the EcoQuest Radiant Catalytic Ionization Cell are presented in Figure 1. Exposure to ozone levels of 0.02 ppm for 2 h reduced all microbial populations tested by at least 0.7 log CFU/cm². Longer exposure times resulted in greater reductions, with the greatest reductions found after 24 h exposure. After 24 h exposure, mean microbial reductions for each organism were as follows: *S. aureus* (1.85 log CFU/cm²), *E. coli* (1.81 log CFU/cm²), *Bacillus* spp. (2.38 log CFU/cm²), *S. aureus* met^r (2.98 log CFU/cm²), *Streptococcus* spp. (1.64 log CFU/cm²), *P. aeruginosa* (2.0 log CFU/cm²), *L. monocytogenes* (2.75 log CFU/cm²), *C. albicans* (3.22 log CFU/cm²), and *S. chartarum* (3.32 log CFU/cm²).

Reductions in microbial populations following treatment of stainless steel coupons with the EcoQuest Breeze AT Ozone generator are shown in Figure 2. Reductions of at least 0.2 and 0.4 log CFU/cm² were observed after 2 and 6 h of ozone exposure, respectively. After 24 h exposure, mean reductions for *C. albicans* and *S. chartarum* were 1.48 and 1.32 log CFU/cm², respectively.

The EcoQuest Radiant Catalytic Ionization Cell and EcoQuest Breeze AT Ozone generators reduced microbial populations on stainless steel surfaces within 2 h under ambient conditions, with greater reductions associated with longer exposure times. The Radiant Catalytic Ionization Cell was more effective than the Breeze AT Ozone Generator at reducing microbiological populations at shorter exposure times of 2 and 6 hours. This study demonstrated that ozone gas has the potential to be an effective surface disinfectant for use in food processing applications. Testing is currently ongoing to evaluate non-treated controls. Phase II of the project, scheduled to be completed by the end of this year, will evaluate the effectiveness of the system for eliminating airborne contamination using the same microorganisms and oxidative technologies.

REFERENCES

- Mork, D.D. 1993. *Removing sulfide with ozone*. Water Contamination & Purification. 34-37.
- U.S. Food and Drug Administration [FDA] 2004. *Recommendations to processors of apple juice or cider on the use of ozone for pathogen reduction purposes*. Accessed 27 July 2005 at <http://www.cfsan.fda.gov/~dms/juicgu13.html>.

Fig. 1 Decontamination of highly polished stainless steel surfaces using the EcoQuest Radiant Catalytic Ionization (ActivePure) Cell

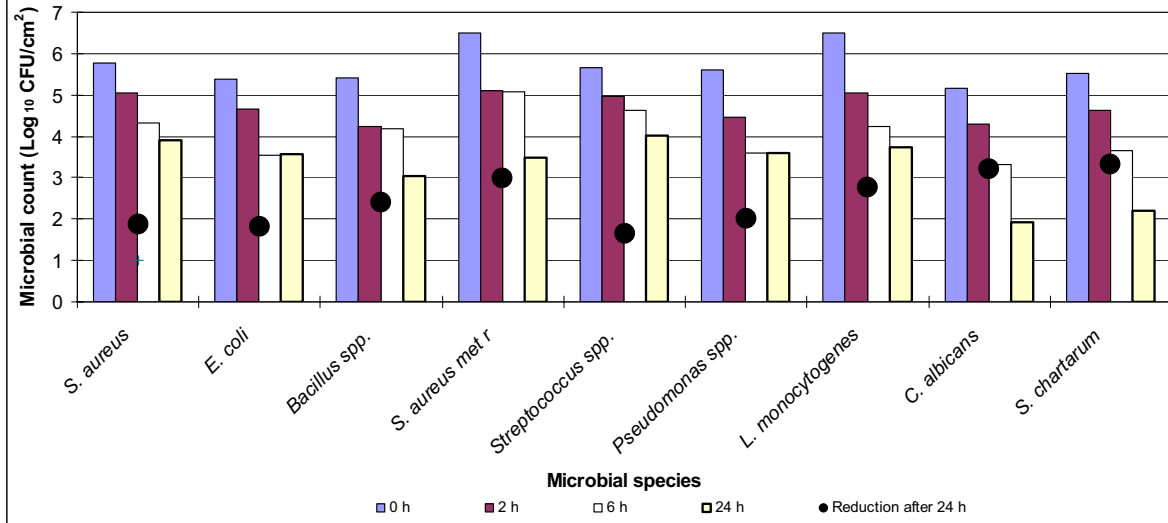


Fig 2. Ozone decontamination on highly polished stainless steel surfaces using the EcoQuest Breeze AT Ozone generator

