Executive Summary
Kansas State University Testing
Biological Reduction through Photocatalysis and Ozone

Summary:
Testing has been performed at the Kansas State Food Science Institute in the Department of Animal Sciences & Industry, Kansas State University in Manhattan Kansas under the direction of Dr. James Marsden, Regent's Distinguished Professor of Meat Science. Kansas State is of America's foremost Universities for animal science and Dr. Marsden is known around the world as one of the top researchers and experts in food safety.

Ten of the most deadly forms of mold, fungi, bacteria and virus were subjected to a new and innovative Photocatalytic Reactor. These nine organisms were placed on a piece of stainless steel inside a test chamber and the PCO cell was turned on for 24 hours. Test results showed a 24-hour reduction ranging from 96.4% to 99.9%.

This testing validates the effectiveness and speed which this PCO cell is able to treat the indoor environment using a natural process at safe levels of oxidation.

Discussion:
With most indoor airborne contaminants originating on surfaces, any efforts to control biological contamination in the indoor environment must address surfaces. Microorganisms such as Mold, Bacteria and Viruses thrive on surfaces in the presence of moisture, and for this reason the food industry has focused on controlling and eliminating pathogens in food contact areas.

Dr. Marsden has dedicated his life to improving food safety through understanding and controlling the spread of biological contamination. Marsden's research has recently focused on the use of advanced Photocatalysis, a technology which develops oxidizers which actively reduce airborne and surface pathogens.

Nine microorganisms were chosen for analysis. Three samples of each microorganism were prepared and placed on a stainless steel surface, allowing analysis at 2 hours, 6 hours and 24 hours of exposure. The test organisms included:

- Staph *(Staphylococcus aureus)*
- MRSA *(Methycillin Resistant Staphylococcus aureus)*
- E-Coli *(Escherichia coli)*
- Anthrax family (*Bacillus* spp.)
- Strep *(Streptococcus* spp.)
- *Pseudomonas aureuginosa*

- *Listeria monocytogenes*
- *Candida albicans*
- Black Mold *(Stachybotrys chartarum)*

These organisms were subjected to air which was circulating through a proprietary photo catalytic reactor. Multiple parameters were monitored including temperature and humidity. The UV Lamp in the photo catalytic cell was positioned in the supply duct to insure there was no effect from the UVGI produced by the lamp. Understanding that Ozone is one of the oxidizers produced in this Photocatalytic process and the health concerns from exposure to excessive levels of ozone, the ozone level was monitored and never exceeded 20 parts per billion, well below EPA maximum level for continuous exposure.

In addition to the test chamber treated with PCO and the corona discharge ozone generator, a control chamber was set up to account for natural decay of the test organisms. Because some biological pathogens die-off on their own when exposed to air, any reputable study must account for such reductions. The test results shown in the report are the reductions in viable organisms with respect to the control sample.

The test results were astounding. After 24 hours of exposure the nine organism's viability was reduced between 96.4% and 99.9%. It should be noted that the double blind study accounted for natural decay. What was even more surprising to the researchers was how fast PCO reduced the pathogens. At the 2-hour sample the average reduction was well over 80%. At the 6-hour sample the average reduction was well over 90%.

An additional test was performed using a corona discharge ozone generator (Breeze AT) against *Candida albicans* (yeast) and *Stachybotrys chartarum* (black mold) at 50 parts per billion (the level deemed safe by the US EPA, OSHA and other international health & safety organizations). This test showed the ability of safe levels of ozone to reduce microbial contamination. It should be noted that although results showed the effectiveness of this safe level of ozone, it also showed that ozone alone is not as effective as the multiple oxidizers produced by the advanced Photocatalytic Oxidation device. One of the multiple oxidizers the PCO cell produces is ozone but at an ozone level two to five times lower than using ozone alone.

This test report has been peer reviewed and was published in the Journal of Rapid Methods & Automation in Microbiology **15** (2007) 359–368.

EFFICACY OF PHOTO CATALYTIC OXIDATION CELL AND OZONE AT REDUCING MICROBIAL POPULATIONS ON STAINLESS STEEL SURFACES

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Accepted for Publication July 11, 2007

ABSTRACT

The improvement of disinfection technology for contact surfaces in health care, food processing, schools and residential environments is critical for the control and prevention of disease-causing microorganisms. Historically, both ozone-and peroxide-based technologies have been used as disinfectants in numerous applications. This study determined the potential use of oxidative gases, including ozone and peroxide, generated by the Photo Catalytic Oxidation (PCO) cell for the inactivation of Escherichia coli, Listeria monocytogenes, Streptococcus pneumoniae, Pseudomonas aeruginosa, Bacillus globigii, Staphylococcus aureus, Candida albicans and Stachybotrys chartarum on stainless steel surfaces. In addition, the ozone generator was evaluated for the inactivation of C. albicans and S. chartarum on stainless steel surfaces at diverse contact times in a controlled airflow cabinet. Results showed that oxidative gases produced by the PCO cell reduced all microorganisms tested by at least 90% after a 24 h exposure on stainless steel surfaces. The PCO cell was more effective at reducing microbial counts for shorter exposure times than was the ozone generator.

PRACTICAL APPLICATIONS

The purpose of this study was to give an accurate evaluation of the Photo Catalytic Oxidation technology for disinfection of environmental contact

surfaces. When used properly and safely, this technology can provide a cost-effective means for eliminating environmental microorganisms such as

*Bacillu*s *globigii*, *Staphylococcu*s *aureus*, *Candid*a *albicans*, *Stachybotry*s *chartarum*, *Pseudomona*s *aeruginosa*, *Escherichi*a *coli*, *Streptococcu*s *pneumonia*e and *Listeri*a *monocytogene*s in industries such as food processing and health care.

INTRODUCTION

Microbial contamination of indoor air represents a major public health problem and a potential source for sick building syndrome. For example, certain species of molds and bacteria may cause health concerns in homes, schools, offices and health care facilities (Hota 2004). In addition to being unattractive to see and smell, molds also give off spores and mycotoxins that cause irritation, allergic reactions or diseases in immune-compromised individuals (Banfleth and Kowalski 2005).

The term *nosocomia*l *infectio*n refers to an infection that is acquired from the hospital or a health care facility (Chotani *e*t *al*. 2004). Environmental contamination has produced devastating consequences in these facilities, resulting in the morbidity and mortality of tens of thousands of patients every year. Persons who visit hospitals, nursing homes or health clinics have a risk of acquiring an infection as a result of their stay (Tilton 2003). It is estimated that approximately one patient in 10 acquires an infection as a result of an extended visit in one of these health care facilities (Tilton 2003). Nosocomial-acquired infections are responsible for approximately 100,000 deaths with an annual cost approaching $29 billion (Kohn *e*t *al*. 1999).

Nosocomial infections have a number of potential causes that promote the spread of diseases. Common health care surfaces such as countertops, beddings, bedpans and medical devices can all be used to transmit and spread diseases from one person to another (Hota 2004). Under hectic and stressful conditions, these surfaces can become easily contaminated, often by overworked employees. Cutbacks in staffing at health care facilities because of budget constraints have placed a greater burden on health care facilities to find ways to remediate contaminants with limited resources (Chotani *e*t *al*. 2004). Older and poorly designed buildings may harbor contaminants that are not easily eliminated using conventional disinfection methods. Studies have shown that microorganisms such as *Staphylococcu*s *aureu*s and *Candid*a *albican*s survive in environmental reservoirs found in health care facilities (Hota 2004).

Food and beverage industries face multiple issues when it comes to

producing a safe, wholesome product. Food pathogens such as *Escherichi*a *col*i 0157:H7, *Listeri*a *monocytogene*s and *Salmonell*a spp. have been a growing concern throughout the years. In addition, processors are concerned about spoilage microorganisms which shorten shelf life and cost companies millions of dollars every year in spoiled products. The areas impacted include the meat, seafood, poultry, produce, baking, canning and dairy industries. The United States Department of Agriculture has estimated the costs associated with foodborne illnesses to be about $2.3 billion and $4.6 billion a year for children and adults, respectively (USDA 2001), in addition to the billions of dollars lost every year because of spoiled products, which must be disposed of or sold at lower values. Reducing pathogens and additional microbial contamination on food contact surfaces decreases cross contamination, improving the quality and shelf life of food products (Kusumaningrum *e*t *al*. 2003). Disinfection and microbiological control measures that efficiently eliminate or diminish microbial counts from every area of food plants are an unquestionable industry investment.

As a disinfectant, ozone has a remarkable ability to oxidize substances. When ozone comes in contact with organic compounds or bacteria, the extra atom of oxygen destroys the contaminant by oxidation. Ozone decomposes to oxygen after being used, so no harmful by-products result (Purofirst 2000). Ozone's oxidation potential is higher than chlorine, 2.07 and 1.36, respectively. Ozone disinfects substances such as water three to four times more effectively. As it oxidizes a substance, ozone literally destroys the substance's molecule leaving virtually no residue behind (Fink 1994).

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 Food and Drug Administration (FDA) approved the use of ozone as a sanitizer for food contact surfaces and for direct application on food products (FDA 2001, 2003). Previously, chlorine was the most widely used sanitizer in the food industry despite the fact that ozone may be more effective for disinfection of surfaces than chlorine. Chlorine is a common disinfectant used in meat processing and is effective and safe when used at proper concentrations. Chlorine, also known by its chemical name sodium hypochlorite, is a halogen-based chemical that is corrosive to stainless steel and other metals used to make food processing equipment. Chlorine can be a significant health hazard to workers when mixed in small amounts with ammonia or acid cleaners producing toxic chlorine gas that can cause massive cellular damage to the exposed nasal passages, trachea and lungs (Gunnarsson *e*t *al*. 1998; Martin *e*t *al*. 2003; Russell *e*t *al*. 2006). In food plants, chlorine may react with meat forming highly toxic and carcinogen compounds called trihalomethanes rendering them lesser-quality products (Cunningham and Lawrence 1977). It can also result in the production of chloroform, carbon

tetrachloride and chloromethane. On the other hand, ozone does not leave any trace of residual product upon its oxidative reaction.

An important advantage of ozone use in food processing is that the product can still be called organic. An organic sanitizer must be registered as a food contact surface sanitizer with the United States Environmental Protection Agency. Ozone has an FDA approval for its use as a sanitizer for food contact surfaces, as well as for direct application on food products.

The use of ozone in food processing has become widely accepted in recent years, and its uses have surpassed surface applications. The FDA (2004) stated, "ozone is a substance that can reduce levels of harmful microorganisms, including pathogenic *E. col*i 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."

The main aim of this study was to evaluate the application of oxidative gases, including low levels of ozone, generated by the Photo Catalytic Oxidation (PCO) cell and the ozone generator against environmental microorganisms such as *E. coli*, *L. monocytogenes*, *Streptococcu*s *pneumoniae*, *Pseudomona*s *aeruginosa*, *Bacilu*s *globigii*, *S. aureus*, *C. albican*s and *Stachybotry*s *chartaru*m on stainless steel surfaces.

MATERIALS AND METHODS

Preparation of Cultures

The following bacteria and fungi cultures were used for the study: *B. globigi*i (American Type Culture Collection [ATCC] #31028, 49822, 49760); *S. aureu*s (ATCC #10832D, 25178, 11987); *C. albican*s (ATCC #96108, 96114, 96351); *S. chartaru*m (ATCC #18843, 26303, 9182); *P. aeruginos*a (ATCC #12121, 23315, 260); *E. col*i (ATCC #27214, 19110, 67053); *S. pneumoniae (ATCC #27945, 29514, 10782); and methicillin-resistant S. aureu*s (ATCC #33591). The cultures were revived using ATCC recommended instructions. *L. monocytogene*s (KSU #56 and 70).

Bacterial, yeast and mold species were independently grown in trypticase soy broth (Difco Laboratories, Detroit, MI) and yeast morphology broth (Difco Laboratories), respectively, to mid-exponential phase followed by a wash and resuspension in 0.1% peptone water. The microbial cultures were combined by species type to ca. 10^8 cfu/mL.

Preparation of Samples and Treatment

The microbial species used to validate the ozone generators were tested as microbial cocktails inoculated onto 6.3 ¥ 1.8 cm, #8 finish stainless steel coupons (17.64 cm^2 double-sided area). Four stainless steel coupons were dipped per microbial inoculum and vortexed 15 s, optimizing microbial dispersion. Binder clips (Universal Brand, Des Plaines, IL), sterilized by autoclaving, were used to hang each stainless steel coupon from a cooling rack for 1 h until dryness in a laminar flow biohazard hood. The initial microbiological populations attached to the stainless steel coupons were in the range of $10^5 – 10^6$ cfu/cm^2. The inoculated stainless steel coupons were transferred to a controlled airflow test cabinet (Mini-Environment Enclosure, Terra Universal, Anaheim, CA) at 26C, 46% relative humidity (ambient conditions) and treated using the PCO cell for 0, 2, 6 and 24 h. The ozone generator was evaluated separately for treatment periods of 0, 2, 6 and 24 h. During the evaluation of the ozone generator, ozone levels were monitored using a Model 500, Aeroqual (Auckland, New Zealand). The ozone levels in the chamber during treatment with the ozone generator were maintained at 0.02 ppm. Nontreated inoculated coupons were evaluated after 0 and 24 h as negative controls.

Sampling

At the end of the ozone contact time, the coupons were placed into 30 mL of 0.1% peptone water and vortexed for 30 s; the samples were serially diluted and plated on trypticase soy agar (Difco Laboratories) for bacterial recovery. Yeast and mold cultures were plated on potato dextrose agar (Difco Laboratories) and cornmeal agar (Difco Laboratories), respectively. The colony-forming units per square centimeter were estimated after 24 h (35C) or 5 days (30C) of incubation for bacteria, yeasts or molds, respectively.

RESULTS

Surface testing to evaluate nontreated control counts is shown in Fig. 1. Microbial reductions on negative controls after 24 h for *S. aureu*s were 0.68 log cfu/cm^2, *E. col*i (0.27 log cfu/cm^2), *Bacillu*s spp. (0.35 log cfu/cm^2), *S. aureu*s (0.47 log cfu/cm^2), *Streptococcu*s spp. (0.31 log cfu/cm^2), *P. aeruginos*a (0.52 log cfu/cm^2), *L. monocytogene*s (0.39 log cfu/cm^2), *C. albican*s (0.45 log cfu/cm^2) and *S. chartaru*m (0.30 log cfu/cm^2). Reductions on non-

treated controls after 24 h ranged from 0.3 to 0.6 log cfu/cm^2.

Reductions in microbial counts on #8 finish stainless steel coupons produced by the PCO cell after 0, 2, 6 and 24 h exposures are presented in Fig. 2. Exposure to ozone levels of 0.02 ppm reduced all microbial populations tested by at least 0.7 log cfu/cm^2 in all microorganisms tested after just 2 h. Longer exposure times resulted in greater reductions with the greatest

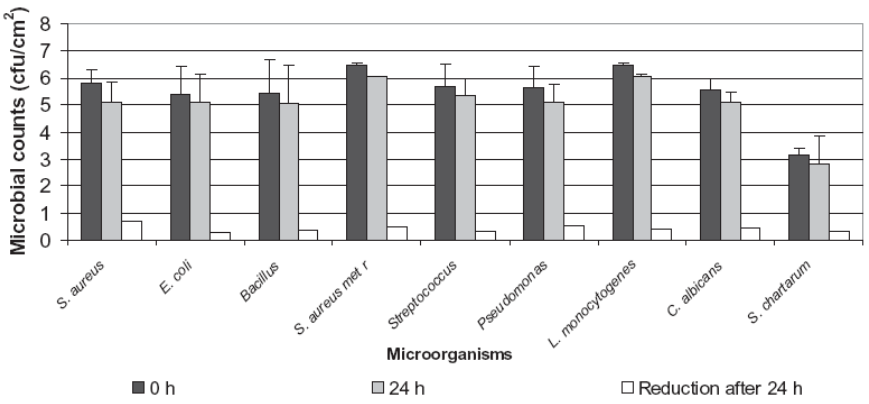

FIG. 1. MICROBIAL SURVIVAL AFTER 24 H INOCULATION ON STAINLESS STEEL COUPONS

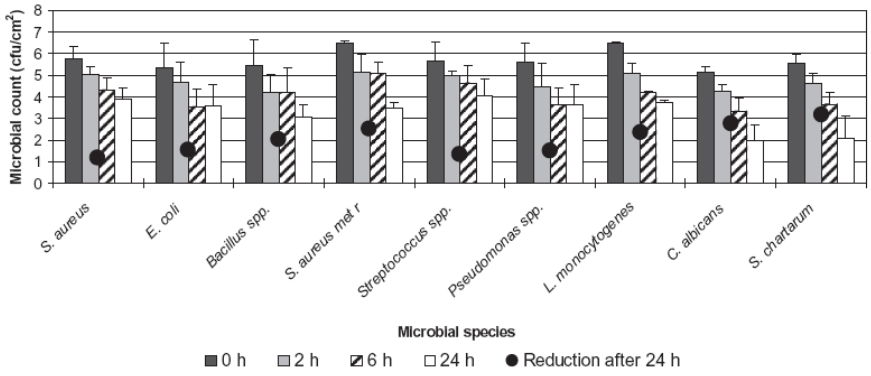

FIG. 2. DECONTAMINATION OF HIGHLY POLISHED STAINLESS STEEL SURFACES USING THE ECOQUEST RADIANT CATALYTIC IONIZATION CELL

reductions found after 24 h exposure. The microbial total reduction mean counts after 24 h exposure for *S. aureu*s were 1.17 log cfu/cm^2, *E. col*i

(1.53 log cfu/cm^2), *Bacillu*s spp. (2.02 log cfu/cm^2), methicillin-resistant *S. aureu*s (2.50 log cfu/cm^2), *Streptococcu*s spp. (1.33 log cfu/cm^2), *P. aeruginos*a (1.48 log cfu/cm^2), *L. monocytogene*s (2.35 log cfu/cm^2), *C. albican*s (2.75 log cfu/cm^2) and *S. chartaru*m (3.16 log cfu/cm^2). Reductions were calculated by taking 0–24 h counts + reduction after 24 h negative controls.

Results of microorganisms tested against the ozone generator are shown in Fig. 3. Exposure to ozone levels of 0.02 ppm resulted in reductions of at least 0.2 and 0.4 log cfu/cm^2 after 2 and 6 h of ozone

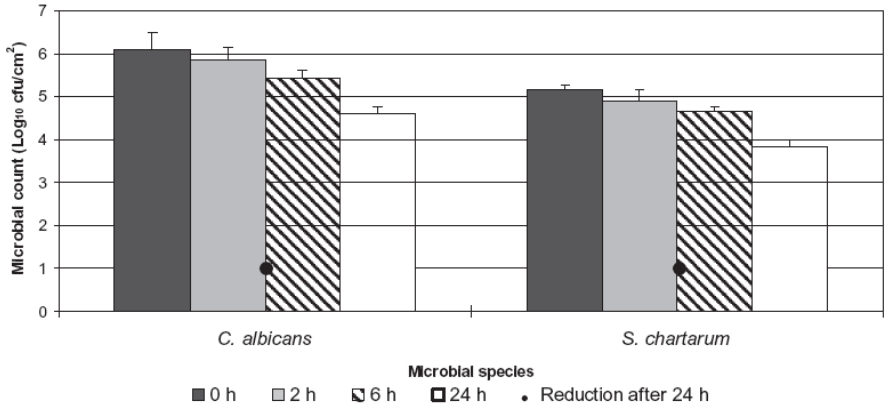

FIG. 3. OZONE DECONTAMINATION ON HIGHLY POLISHED STAINLESS STEEL SURFACES USING THE ECOQUEST BREEZE AT OZONE GENERATOR

exposure. After 24 h of exposure (calculated as described earlier), the *C. albican*s and *S. chartaru*m reduction means were 1.02 and 1.01 log cfu/cm^2, respectively.

DISCUSSION

Oxidative gases such as ozone have been used by industry for many years and in numerous applications such as odor control, water purification and as disinfectants. Ozone can oxidize organic substances such as bacteria and mildew, sterilize the air and destroy odors and toxic fumes (Mork 1993). An area where ozone technology may be utilized more in the future is in removing environmental contaminants. It has been reported that ozone levels of less than 9 ppm are all that is needed to remediate sick buildings or for professional disinfection (Khurana 2003). In this study, levels of 0.02 ppm and less were found to have an affect at reducing populations of environmental

microorganisms.

The application of this type of technology may be most beneficial in areas where environmental contamination is of growing concern such as in health care. Fear of nosocomial infection in chronic care facilities is a problem because of the extended time patients are exposed to the risk of infection. The anticipated increase in the elderly population in the next several decades makes prevention of infection in long-term care facilities a priority (Nicolle 2001).

Ozone applied in the food industry has proven to be a powerful, broad-spectrum antimicrobial agent that is effective against bacteria, fungi, viruses, protozoans and bacterial and fungal spores. A study by Kim *e*t *al*. (1999) found that an ozone rinse of just 1.3 ppm for 5 min produced a greater than 99.9% reduction in psychrotrophic and mesophilic bacteria on lettuce. The ozone technology evaluated in this study would give processors a resource for controlling environmental contaminants, adding to their overall sanitation program.

To our knowledge, it is the first time a study has been conducted to test microbial reductions on stainless steel surfaces by exposure to oxidative gases and gaseous ozone. In this study, a low concentration of ozone (0.02 ppm) reduced all microorganisms tested by at least 90% after a 24 h exposure on stainless steel surfaces.

Short exposure times (2 h) to ozone levels of 0.02 ppm reduced all microbial populations tested by at least 0.7 log cfu/cm^2 in all microorganisms tested. It has been reported that the antimicrobial activity of ozone is based on its strong oxidizing effect, which damages the cell membrane (Pope *e*t *al*. 1984). Ozone kills bacteria within a few seconds by a process known as cell lysing. Ozone molecularly ruptures the cellular membrane, disperses the cell's cytoplasm and makes microbial survival impossible. Because of these actions, microorganisms cannot develop ozone-resistant strains, eliminating the need to change biocides periodically (Pope *e*t *al*. 1984).

The PCO cell and ozone generator reduced microbial populations on stainless steel surfaces within 2 h under ambient conditions, with greater reductions associated with longer exposure times. The PCO cell was more effective than the ozone generator at reducing microbiological populations at shorter exposure times of 2 and 6 h. This study demonstrated that the low levels of oxidative gases produced by the PCO cell have the potential to be an effective surface disinfectant tool for use in food processing, sick building remediation and health care applications.

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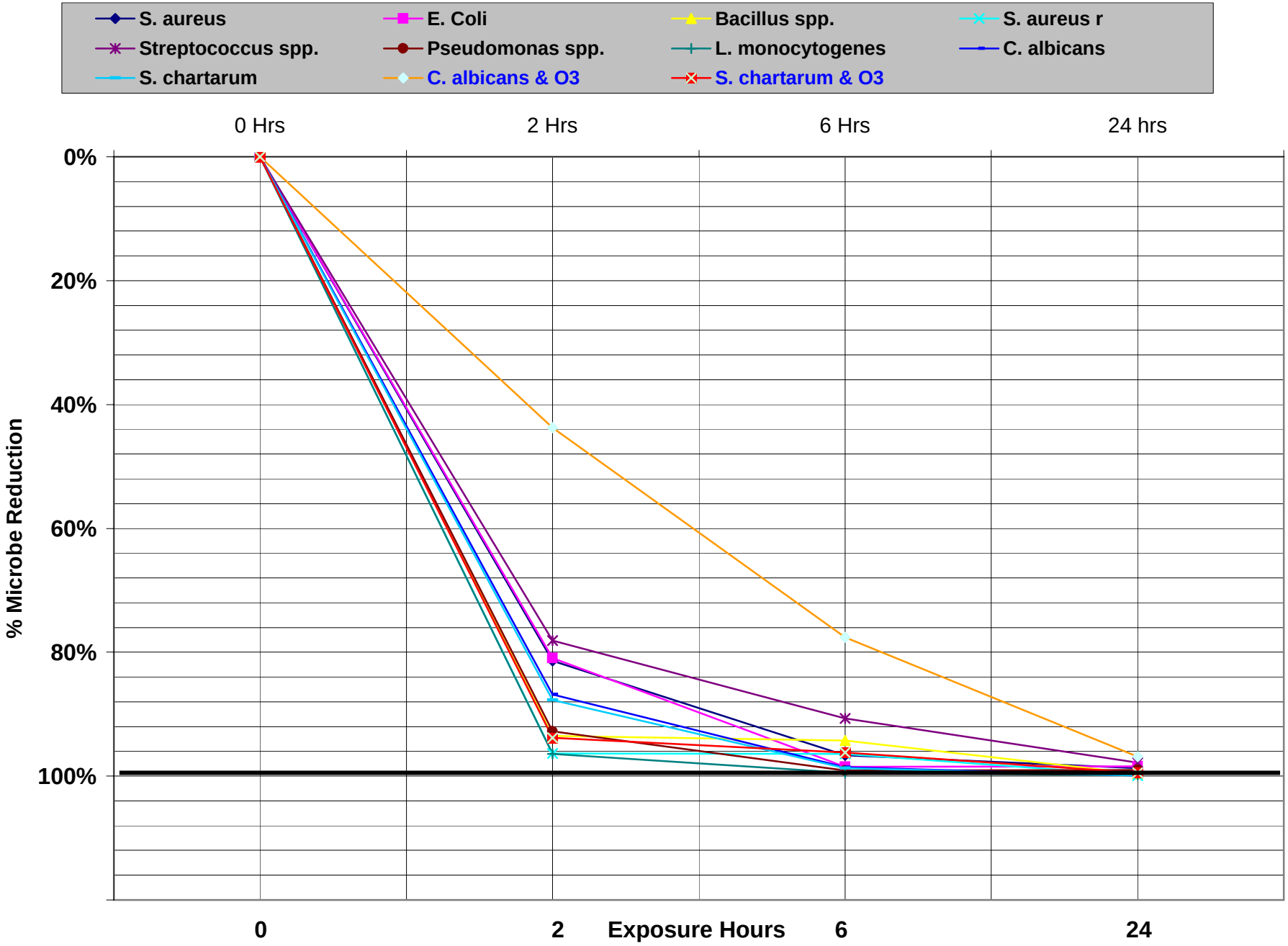
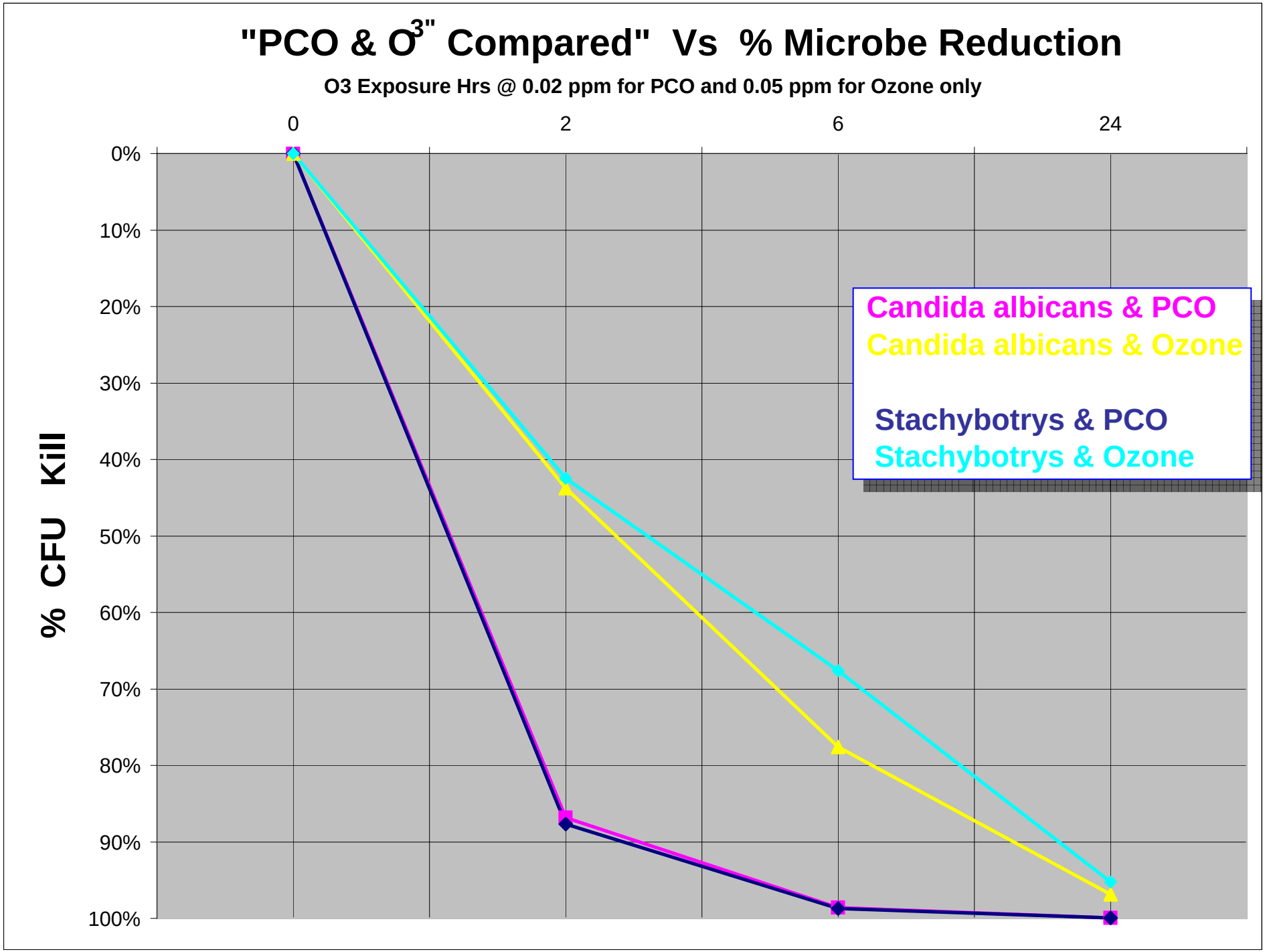
Kansas State University Microbe Reduction on Surfaces Using "PCO & Ozone" Technologies




"PCO & O3" Compared" Vs % Microbe Reduction
O3 Exposure Hrs @ 0.02 ppm for PCO and 0.05 ppm for Ozone only
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70%
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Candida albicans & PCO
Candida albicans & Ozone
Stachybotrys & PCO
Stachybotrys & Ozone