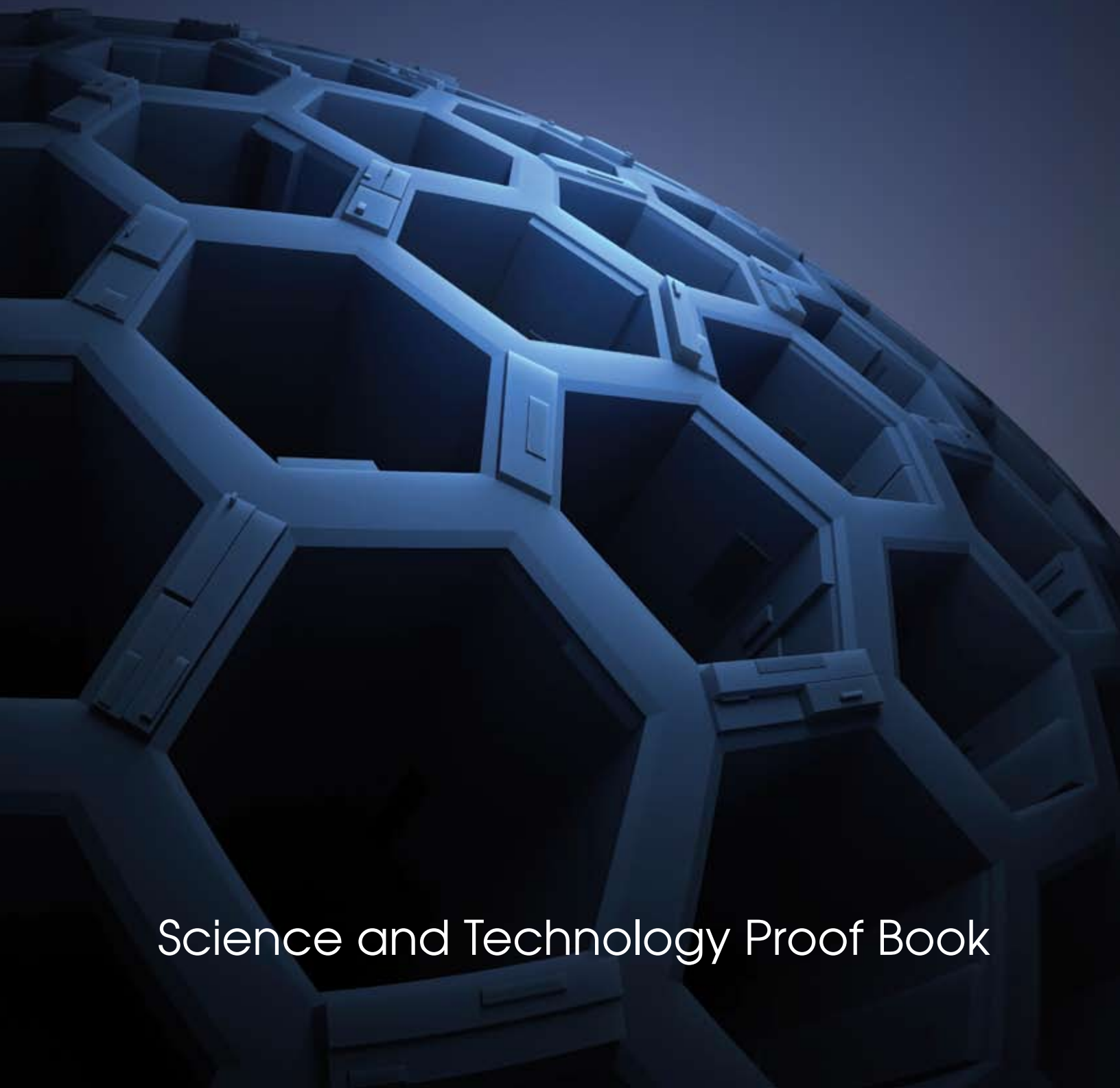
EcoQuest International



Science and Technology Proof Book

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For Immediate Release

News From the Space Foundation

EcoQuest International Awarded Certified Space Technology™ Status

Colorado Springs, CO (June 30, 2004) – The Space Foundation announced today that EcoQuest International's Fresh Air purification product has been officially recognized as a Certified Space Technology™.

Fresh Air by EcoQuest uses technology originally developed in cooperation with NASA to clean the air in spacecraft by removing airborne pathogens. Titanium Dioxide (Ti02), a photocatalytic substance, was discovered to reduce hydrocarbons in enclosed spaces. The Fresh Air product uses a similar application of Ti02, in a UV activated photocatalytic system, to effectively purify the air indoors.

According to a December 1998 *Wall Street Journal* article, carpeting, poorly ventilated fireplaces, mold, bacterial toxins, dust mites . . . an almost endless collection of highly allergenic products have invaded our homes, and we have sealed them in with deadly precision. "People need products like our Fresh Air because they help solve significant problems," stated Michael Jackson, EcoQuest President.

"EcoQuest has been awarded use of the Certified Space Technology™ seal because they have effectively applied space based technology to address the real and growing concern of indoor air pollution and improve the quality of life for people here on Earth," said Kevin C. Cook, Space Foundation Director of Space Awareness Programs.

- more -

"The Space Foundation is committed to recognizing and supporting the efforts of companies like EcoQuest," said Cook. EcoQuest is the most recent member of our growing list of Space Certification program partners including industry leaders such as Tempur-Pedic® Sleep Surfaces, X-1R Advanced Lubricants, and Outlast Phase Change Materials."

About EcoQuest International

EcoQuest is dedicated to improving the spaces where people live by providing innovative products and services designed to enhance and improve the quality, safety, convenience, and beauty of living indoors. For more information about EcoQuest International and the Fresh Air product, visit www.ecoquest.com.

About The Space Foundation

Founded in 1983 and headquartered in Colorado Springs, CO, the Space Foundation is a non-profit organization whose mission is to vigorously advance and support civil, commercial and national security space endeavors and educational excellence.

The Space Foundation, in cooperation with NASA, established the Space Certification Program and the Space Technology Hall of Fame to recognize innovators who transform technology, originally developed for space use, into commercial products, to increase public awareness of the benefits of space transfer technology, and to encourage further innovation.

For more information about the Space Foundation, the Space Technology Hall of Fame and the Space Certification Program, visit www.spacefoundation.org.

FOR IMMEDIATE RELEASE

ECOQUEST RECEIVES TOKENS OF APPRECIATION FROM DEPARTMENT OF DEFENSE

Greeneville, TN – EcoQuest International, an indoor environmental technology company, recently received two Department of the Army DSS-W coins in appreciation for the donation of air purification equipment following the attack on the Pentagon on September 11, 2001.

"Like the rest of the country, our company saw the destruction on television and wondered how we could help," said EcoQuest founder and CEO Mike Jackson. "With some help from contacts within the Government, we learned that the smoke and fire damage left lingering odors in the building. That was tough, because people still had to go work in their offices the next day. That's where we could help."

EcoQuest's Fresh Air technology is scientifically proven to eliminate smoke and odors in the air. By installing the equipment, employees at the Pentagon immediately noticed a difference.

"We received a letter thanking us for the donation, along with the Defense Supply Service coins. That meant a lot to us," Jackson shared. "But a month later, when we received another letter from an Army Colonel letting us know the products were really helping, we were most touched. As he put it, he 'witnessed the tremendous improvement in the air quality in the offices.' That meant a lot to us, because we really wanted to help make that environment more livable."

About EcoQuest International

EcoQuest International, a healthy living technology company, is the world's largest distributor of "Certified Space" indoor air purification and water purification systems. EcoQuest's flagship Fresh Air technology, proven in university studies to eliminate up to 99.99% of mold, viruses, and bacteria on surfaces*, recreates natural processes to improve the air indoors in more than five million homes and businesses around the globe. Based in Greeneville, TN, EcoQuest employs more than 500 people, and has a sales force of nearly 100,000 active distributors around the globe. For more information, visit www.EcoQuest.com.

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* University testing demonstrates the ability of EcoQuest's ActivePure technology to substantially reduce microbial populations on **surfaces**. Field results may vary based on environmental conditions. These statements have not been evaluated by the FDA. These products are not intended to diagnose, treat, cure, or prevent any disease.

FOR IMMEDIATE RELEASE

ECOQUEST INTERNATIONAL INTRODUCES SPACE TECHNOLOGY TO CLEAN INDOOR AIR AT 2005 INTERNATIONAL BUILDER'S SHOW / NEXTGEN HOME.

Orlando, FL – It used to be that when you heard about air pollution, you thought only of the outside air we breathe; however, many people are unaware that the air **INSIDE** their homes is potentially even **MORE** polluted.

The advent of modern building techniques has produced greater energy savings and stronger homes, but at the cost of the natural air purification processes that occur outdoors. Until recently, the problem of indoor air pollution was virtually unknown. For almost 20 years, a company headquartered in East Tennessee has been educating consumers while providing innovative technology solutions to combat the problems of poor air and water quality.

The company is called EcoQuest International, and the Fresh Air by EcoQuest product line is on the cutting edge of air purification technology. Duplicating natural processes, EcoQuest's solutions do not offer a cover-up for the problem – they work to eliminate it. EcoQuest's SynAirG process uses the same technology used by NASA to clean the air in the space program. In fact, EcoQuest's main product line has received "Certified Space Technology" accreditation from the Space Foundation.

EcoQuest's air products have also been given the Handy Man Club of America's Seal of Approval, and EcoQuest's products are the premier air purification technology chosen for the NextGen 2005 Home of the Future project at the 2005 International Builders' Show in Orlando.

EcoQuest markets their products directly to consumers through an extensive direct marketing network, and the EcoQuest team believes that partnering with homebuilders makes perfect sense. With a patented system known as DuctwoRx, homeowners can begin to enjoy the benefits of clean, fresh air the day they move into their new home. DuctwoRx is easily and inexpensively installed by the builder into the HVAC system and immediately begins to reduce airborne pollutants as the air is being circulated through the home.

According to Mike Jackson, President and Founder of EcoQuest International, the International Builders' Show will give homebuilders and others in the home building industry the opportunity to see the products in use, first hand, and experience the Fresh Air difference. "Our mission is to help people live better; it is at the heart of what we do."

FOR IMMEDIATE RELEASE
Environmental Health News

FRESH AIR BY ECOQUEST REDUCES PRESCHOOL ABSENTEEISM BY 75%, CBS NEWS REPORTS

Greeneville, TN – A unique air purifier, Fresh Air by EcoQuest, has helped reduce absenteeism from illness in a Pennsylvania preschool, as reported by CBS Station 21 in Harrisburg.

"Since owners Nancy and Ken Goss installed the system, absenteeism from illness went down more than 75% according to school logs," said Shannon Murphy, director of Goddard School, a preschool and daycare in Mechanicsburg.

"During flu season we counted maybe 25 to 30 kids out during the 3-week period last year," Nancy Goss told WHP-TV reporter Sherry Christian. "This year maybe five to 10 were out in the same period."

Employees and parents noticed a change in the air quality in the school.

"Parents and teachers say they noticed an improvement in the quality of the air right away," Murphy said. "It smells cleaner here."

But the tangible proof, reported Christian, was a reduction in the number of tissues bought for the school.

"Normally I buy cases of tissue at a time," said Murphy. "This last time I bought one case."

Before the system was in place, teacher Rose Gustkey said, "I'd be stuffy and my throat would be sore all the time, but now I feel so much better."

Teacher Sherrie Swartz agreed. “Normally I come in with colds. It has made a difference for my students and me. They’re not using as many tissues.”

“The important thing to understand about our Fresh Air technology is that it is treating environments, not people,” shared EcoQuest Founder Mike Jackson. “Our technology creates healthier spaces, which helps prevent the spread of germs on surfaces.”

Scientific tests at Kansas State University have demonstrated that the use of Fresh Air by EcoQuest kills up to 99.99% of germs on surfaces, including Staphylococcus aureus, Methycillin Resistant Staphylococcus aureus (MRSA), E-coli, Bacillus spp. (anthrax), Streptococcus spp., Listeria monocytogenes, Candida albicans, Stachybotrys chartarum (black mold) and Avian Influenza H5N8.

Dr. James Marsden, Regents Distinguished Professor of Food Safety and Security at Kansas State University, conducted those tests.

“I was continually impressed with the ability of Fresh Air to inactivate bacterial pathogens, viruses, mold spores, and more,” he said.

Part of what makes Fresh Air unique is its advanced “Space Certified” Radiant Catalytic Ionization (RCI) technology. This is the same technology NASA has experimented with to scrub the air on spacecraft with the goal of extending the lifespan of plants in space.

“With a WebMD website reporting that roughly 80% of colds and flu result from contact with germ-contaminated surfaces, it’s easy to understand why CBS is reporting such positive results at the Goddard School,” says Jackson.

About EcoQuest International

EcoQuest International, a healthy living technology company, is the world’s largest distributor of “Certified Space” indoor air purification and water purification systems. EcoQuest’s flagship Fresh Air technology, proven in university studies to eliminate up to 99.99% of mold, viruses, and bacteria on surfaces*, recreates natural processes to improve the air indoors in more than five million homes and businesses around the globe. Based in Greeneville, TN, EcoQuest employs more than 550 people, and has a sales force of nearly 100,000 active distributors around the globe. For more information, visit www.TryFreshAir.com.

* University testing demonstrates the ability of EcoQuest’s ActivePure technology to substantially reduce microbial populations on **surfaces**. Field results may vary based on environmental conditions. These statements have not been evaluated by the FDA. These products are not intended to diagnose, treat, cure, or prevent any disease.

FOR IMMEDIATE RELEASE
Environmental Health News

AIR PURIFIERS FROM TENNESSEE-BASED ECOQUEST USED TO WARD OFF BUGS IN UK HOSPITAL

Greeneville, TN – The BBC has reported that a hospital in Somerset, England, has installed machines manufactured in Greeneville, Tenn., to combat airborne bugs, odors, and pollutants.

Clevedon Hospital wants to keep its record of never having had a case of MRSA (Methicillin-resistant Staphylococcus aureus), a sickness that patients sometimes catch in the hospital. To do this, they installed EcoQuest International's Fresh Air air purifiers. Studies at Kansas State University have proven that the Fresh Air kills up to 99.99 % of germs on surfaces.

Testing in the Somerset hospital began in summer 2006, and officials there have now put them in its wards and minor injuries department.

"We are pleased to reassure our community that we are working hard to protect the hospital and its patients from infection," said Matron Gwen Hobbs in a BBC News report in January 2007.

The hospital said that research has shown them to be particularly effective against MRSA and norovirus, which causes diarrhea and vomiting and is a problem affecting many hospitals, especially in the winter months.

Tests conducted in the U.S. by Dr. James Marsden, Regents Distinguished Professor of Food Safety and Security at Kansas State University, showed that the EcoQuest air purifiers kill up to 99.99% of the bacteria, viruses and mold on surfaces. This includes the norovirus, E. coli, Listeria monocytogens, Streptococcus spp., Psuedomonas aeruginosa, Bacillus spp., Staphyloococcus aureus, Candida albicans, S. chartarum, Avian Bird Flu and more.

EcoQuest's air purification system was used at the Pentagon following 9/11 to combat toxins and pollutants resulting from the tragedy. EcoQuest Products are also used and endorsed by national radio personality Dr. Laura. And Fresh Air by EcoQuest was the chosen product for air purification in the 2005 NextGen House of the Future Project at the Orlando Builders Show.

Part of what makes Fresh Air unique is its advanced "Space Certified" Radiant Catalytic Ionization (RCI) technology. This is the same technology NASA has experimented with to scrub the air on spacecraft with the goal of extending the lifespan of plants in space.

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FOR IMMEDIATE RELEASE
Environmental Health News

KANSAS STATE UNIVERSITY STUDY ON FRESH AIR PUBLISHED ON USDA WEBSITE

GREENEVILLE, TN.—EcoQuest International, a Greeneville, Tenn.-based company that is already a world-leader in air and water purification systems, has teamed with Kansas State University to prove the efficacy of their products. The results of the latest study have been published on the U.S. Department of Agriculture's website.

"This is not an endorsement by the USDA," says EcoQuest Founder Mike Jackson. "It is however very telling about the credibility and substantiation of one of the benefits our products offer. In this study, Fresh Air killed up to 99.99% of viruses, bacteria, and molds on surfaces."

Dr. James Marsden, leader of the food safety research team at Kansas State University, said the process can be likened to a "miniature sun" of ultraviolet energy that interacts with oxygen to create an antimicrobial effect. The research found that the system was effective in reducing several pathogens, including E. coli, Listeria monocytogenes and Staphylococcus aureus.

"EcoQuest has created an advanced purification system based on the same research used by the National Aeronautics and Space Administration to decontaminate spacecraft during long missions," said Marsden, a KSU Regent's

Distinguished Professor. He also serves as the associate director of the National Agriculture Biosecurity Center, located at KSU.

The findings were recently posted on the U.S. Department of Agriculture Web site at http://fsrio.nal.usda.gov/news_article.php?article_id=3483.

The researchers found that the Fresh Air by EcoQuest system removed more contaminating bacteria from stainless steel surfaces at a shorter exposure time than traditional ozone-based methods. The U.S. Food and Drug Administration approved the use of ozone as a sanitizer for food and contact surfaces in 2001.

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FOR IMMEDIATE RELEASE
Environmental Health News

NOROVIRUS ON THE RISE - ECOQUEST PRODUCTS SUPPORT CDC WELLNESS STRATEGIES

Greeneville, TN – *USA Today* recently reported the Center for Disease Control's release of a strategy for combating the increasing incidents of stomach flu in the U.S. These strategies involved, amongst other things, decontaminating linens and keeping surfaces decontaminated. EcoQuest's Fresh Air and LaundryPure products support those strategies.

EcoQuest's Fresh Air product is proven to kill up to 99.99% of germs like Norovirus, on surfaces, in University studies. NSF testing of EcoQuest's LaundryPure product has also demonstrated over a 99.999% kill of germs in the wash. Together, they support a strategy for a healthier living space.

The nasty norovirus, most commonly called "contagious gastroenteritis," is the cause of many cases of the "stomach flu" in the United States. According to experts at the Center for Disease Control (CDC), most cases occur in the winter months, but 2006 saw two to three times as many cases as usual. Experts say that for every person who reports illness, there are two or three who don't.

"The best strategy for containing the virus," says Widdowson, "is cleanliness. Wash hands frequently, and for up to 20 seconds. Wash clothing and bed linen in hot water and dry in a hot dryer. Wear gloves when caring for the sick. And most importantly, disinfect surfaces."

Once the virus is in the house, experts say, it's tough to eradicate. It lingers for days on surfaces like children's toys, telephones, and doorknobs. Not to mention vomit and stool are extremely infectious. The virus can remain in stool for up to three weeks after recovery.

Flu season hits every winter, culminating in February and March. Some years are worse than others, and with increasing population numbers, families are more and more at risk. Add to that warnings about the Avian Bird Flu, and people have cause to worry.

“We can run around with a disinfectant spray bottle and sponge all day,” says Mike Jackson, EcoQuest International’s Founder, “or we can turn on a Fresh Air by EcoQuest. University tests have demonstrated that the use of EcoQuest air purifiers kills up to 99.99% of germs on surfaces.”

Tests conducted in the U.S. by Dr. James Marsden, Regents Distinguished Professor of Food Safety and Security at Kansas State University, showed that the EcoQuest air purifiers kill up to 99.99% of the bacteria, viruses and mold on surfaces. This includes the norovirus, E. coli, Listeria monocytogens, Streptococcus spp., Psuedomonas aeruginosa, Bacillus spp., Staphyloococcus aureus, Candida albicans, S. chartarum, Avian Bird Flu and more.

“Then, you can put your sheets and towels in a washing machine with LaundryPure installed, and you’ve got some additional germ-killing power to help bring peace of mind,” says Jackson.

Part of what makes Fresh Air unique is its advanced “Space Certified” ActivePure (Radiant Catalytic Ionization - RCI) technology. This is the same technology NASA has experimented with to scrub the air on spacecraft with the goal of extending the lifespan of plants in space.

“Our effective air and surface purification technology is combined with silver ions to help kill germs in the wash with LaundryPure,” says Jackson. “It’s quite a package.”

About EcoQuest International

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News Stories

Fresh Air and Freedom

As tens of thousands visit the Liberty Bell this year, they will be breathing rainstorm-fresh air, because the visitor's staging area is protected with Fresh Air DuctworRx.

Special thanks to EcoQuest Business Owner Ray Sears for working with the National Park Service to protect the visitors of this historic landmark.

EcoQuest is providing fresh air to a symbol of American history.

Fresh Air and the Red Cross

EcoQuest teams up with local leaders to combat Southern California wildfire challenges.

As the American Red Cross set up rescue shelters around the area, EcoQuest reached out to donate 75 Breeze Air units for use in the shelters. The units were placed in two large Red Cross Shelters at the Orange Show Fairgrounds in San Bernardino, CA.

"We have a number of Business Owners in the Southern California area and this was just one way we found that we could be of assistance in this terrible time for so many people," Mike Jackson said. "We hope that our purifiers make things just a little bit better for those who are without homes to return to."

The facility is over 30,000 square feet, and is currently housing more than 600 people.

"With this many people in one place, the units worked quickly and efficiently to deal with various odors that had accumulated," a Red Cross worker stated.

A group of EcoQuest Business Owners spent six hours at the location helping set up the purifiers, teaching staff how to use and adjust the settings, while spending time with shelter victims.

The Red Cross did request a personal unit be donated to a woman with special circumstances. She had a small child that was in need of access to an air purifier, due to the poor air quality, but had been unable to purchase one due to her financial situation. Our Business Owners spoke with the woman at the shelter and provided her with a Living Air. The woman tearfully thanked them.

Folks, this is what it's all about. Helping people live better. Our hats go off to those Business Owners who invested their time and money to help make this happen. The Red Cross will keep units to use for future rescue shelter situations.












Ground Zero Museum Guests Breathe Easier With Fresh Air

In New York City, the doors to the Ground Zero Museum opened in remembrance to the tragic events of 9/11.

On display were artifacts recovered from the rubble of the Twin Towers. While the experience of walking through the museum was very emotional, it was also difficult to reflect on the displays due to the lingering effects of that day.

There were terrible odors in the air.

However, through the New York City Fire Department, the curator of the museum discovered Fresh Air by EcoQuest. In 2001, EcoQuest and its Business Owners donated an EcoQuest air purifier to every fire station in New York City, and the surrounding communities, who's brave firefighters were impacted by that day.

Today, Fresh Air is treating the air at the Ground Zero Museum. "When our staff came in the day after Fresh Air was installed, they could tell immediately that the air was cleaner," says Gary Suson, photographer and curator of the museum.

Research Papers - Technology

Executive Summary by Allen Johnston - Chief Technology Officer, EcoQuest International

Kansas State University Testing

Biological reduction on surfaces through photocatalysis and ozone with ActivePure RCI technology.

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 called Radiant Catalytic Ionization (ActivePure). These ten organisms were placed on a piece of stainless steel inside a test chamber and the ActivePure cell was turned on for 24 hours. Test results showed a 24-hour reduction ranging from 96.4% to 100%.

This testing validates the effectiveness and speed which RCI is able to treat the indoor surface 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.

Ten 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 aureuginos
- Listeria monocytogenes
- Candida albicans
- Black Mold (Stachybotrys chartarum)
- Avian Influenza H5N8

These organisms were subjected to air which was circulating through a proprietary photo catalytic reactor called Radiant Catalytic Ionization or RCI (ActivePure). 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.

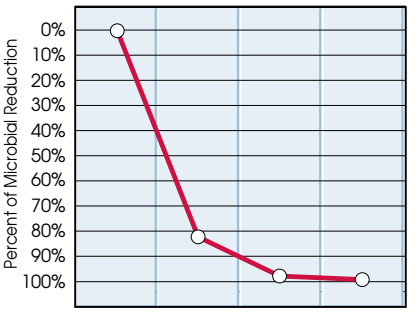
In addition to the test chamber treated with ActivePure and the corona discharge ozone generator, a control chamber was set up to account for natural decay of the test organisms. Because some biological pathogens die-off on their own when exposed to air, any reputable study must account for such reductions. 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 100%. It should be noted that the double blind study accounted for natural decay. What was even more surprising to the researchers was how fast ActivePure 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%.

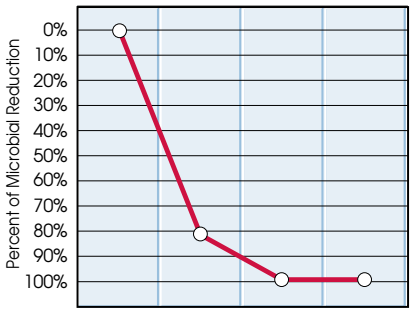
Effects of ActivePure (RCI) Technology

on reducing common bacteria and fungi on **surfaces*** in 24-hour testing.

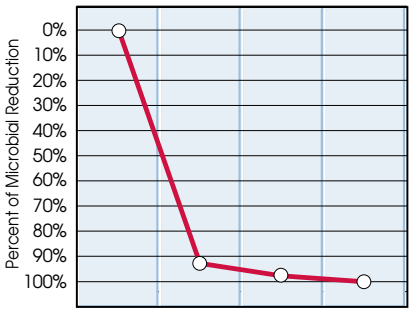
S. aureus Average of two 24-hour tests



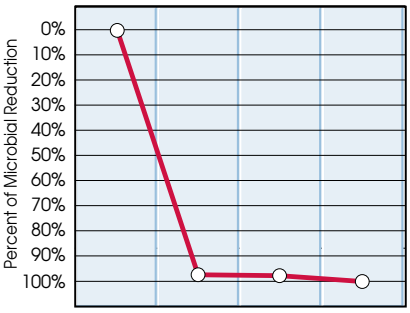
E. Coli Average of two 24-hour tests



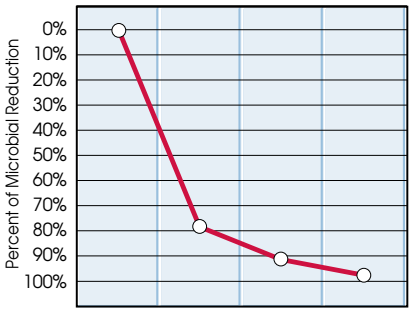
Bacillus spp. Average of two 24-hour tests



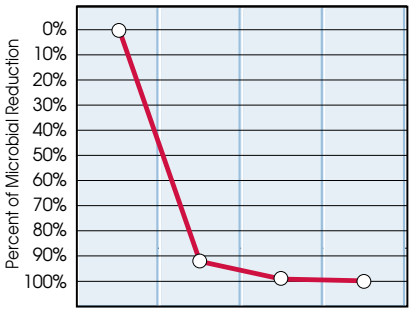
S. aureus r Average of two 24-hour tests



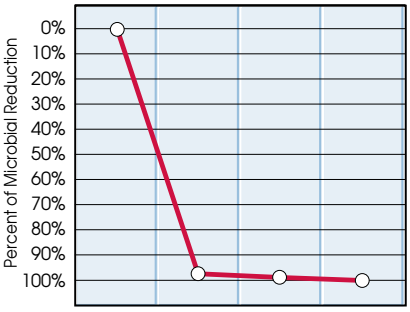
Streptococcus spp. Average of two 24-hour tests



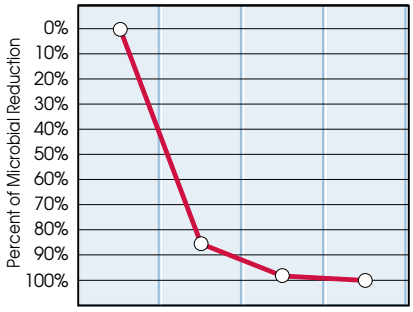
Pseudomonas spp. Average of two 24-hour tests



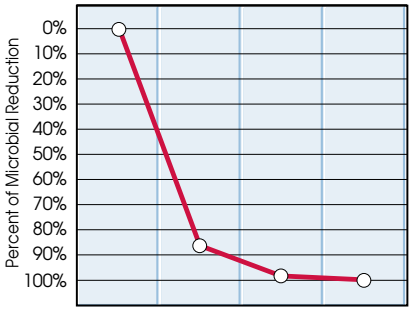
L. monocytogenes Average of two 24-hour tests



C. albicans Average of two 24-hour tests



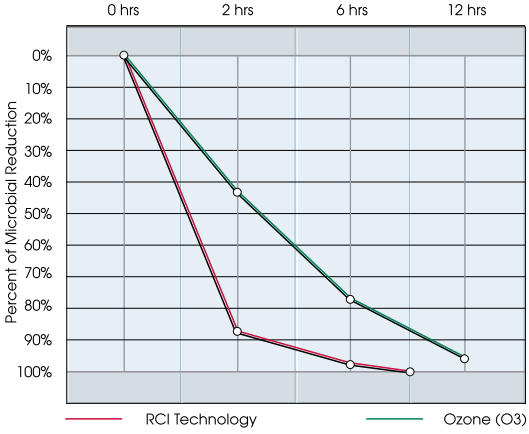
S. chartarum Average of two 24-hour tests



Comparing The Effects of ActivePure (RCI) Technology and Ozone Technology

on reducing common bacteria and fungi on **surfaces*** in 24-hour testing.

C. albicans (Candida albicans) Average of two 24-hour tests



Testing by Kansas State University. Field results may vary based on environmental conditions.

*Scientific testing has demonstrated the use of EcoQuest's ActivePure technology to substantially reduce microbial populations on surfaces – including but not limited to Escherichia coli, Listeria monocytogenes, Streptococcus spp., Pseudomonas aeruginosa, Bacillus spp., Staphylococcus aureus, Candida albicans, and S. chartarum. Field results may vary based on environmental conditions. No claim with respect to airborne microbials is made based on these results. These results have not been evaluated by the FDA. This product is not a medical device intended to diagnose, treat, cure, or prevent any disease.

Efficacy of EcoQuest Radiant Catalytic Ionization (ActivePure) 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 (ActivePure) Cell for the inactivation of Escherichia coli,Listeria monocytogenes,Streptococcusspp.,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 (ActivePure) 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 moncytogenes,* 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 cm^2 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/cm^2. 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/cm^2).

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 metr (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 (ActivePure) 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 (ActivePure) 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.

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

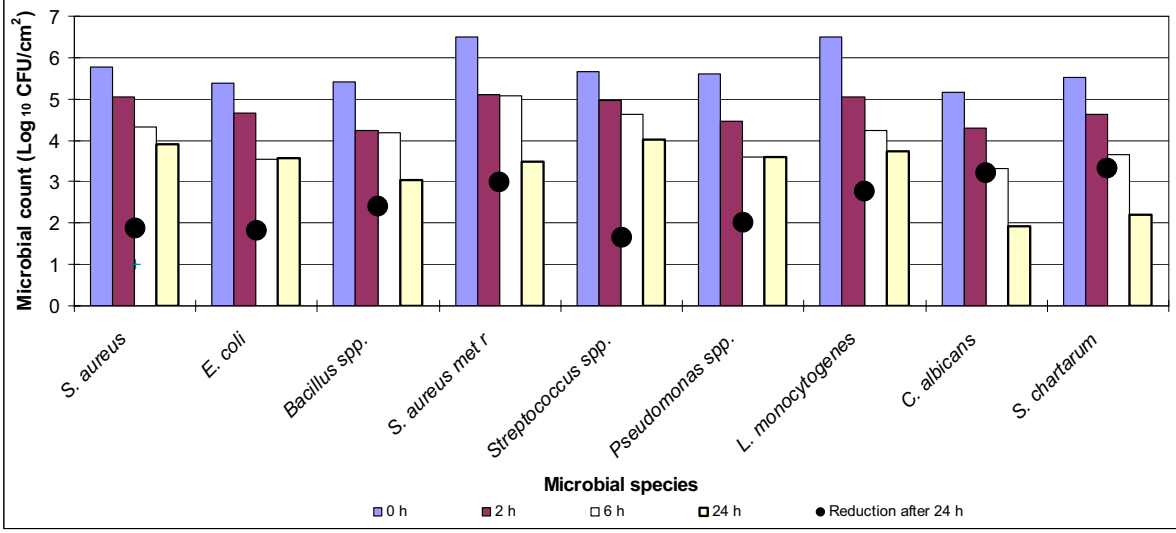
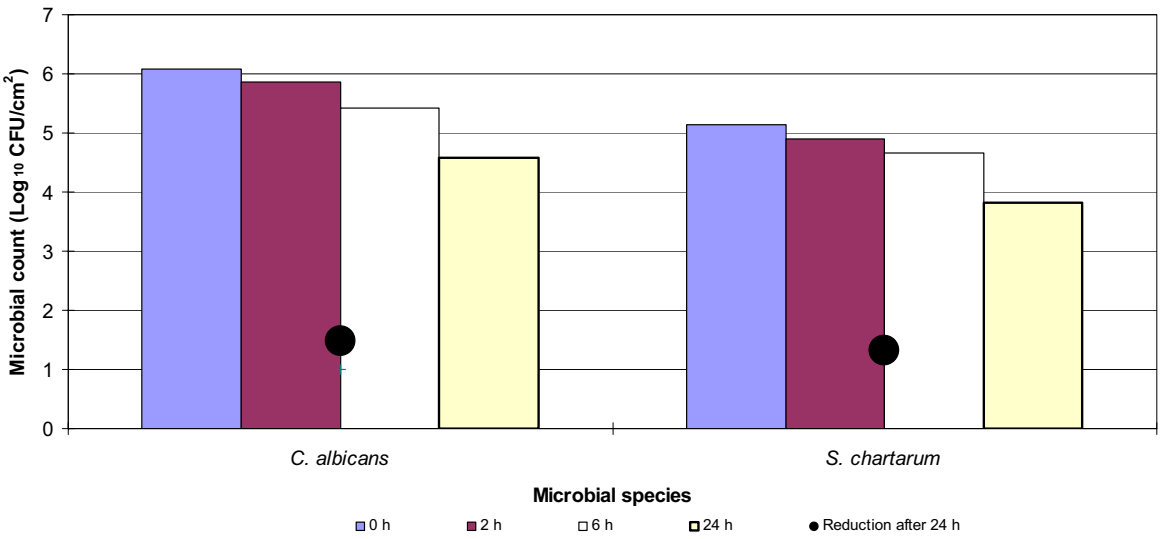
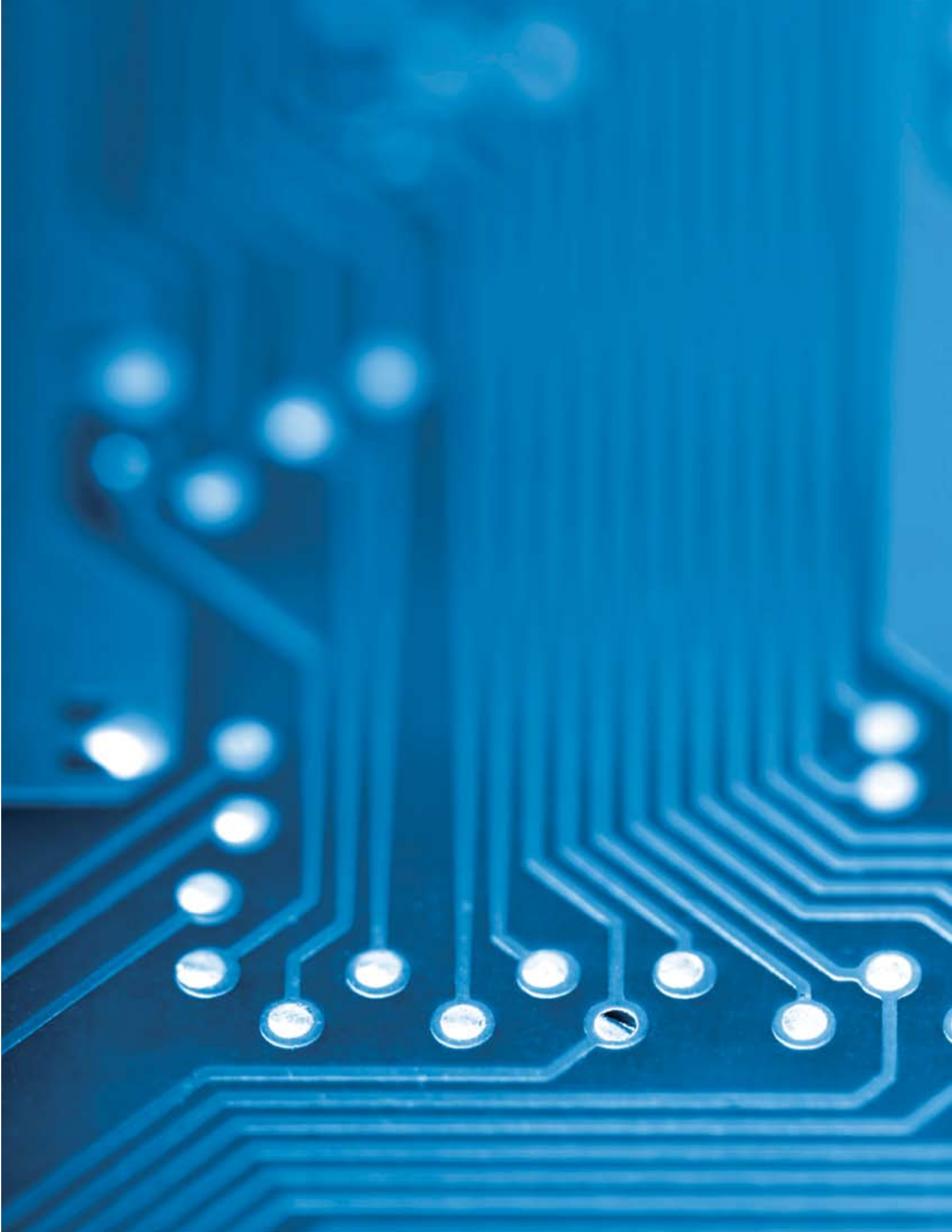


Fig 2. Ozone decontamination on highly polished stainless steel surfaces using the *EcoQuest Breeze AT Ozone* generator



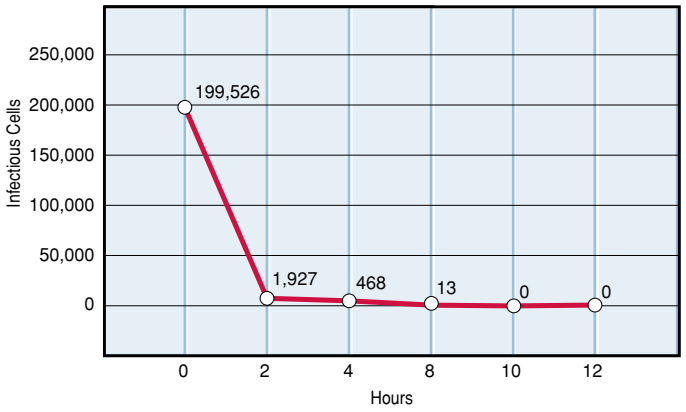


Effects of ActivePure (RCI) Technology

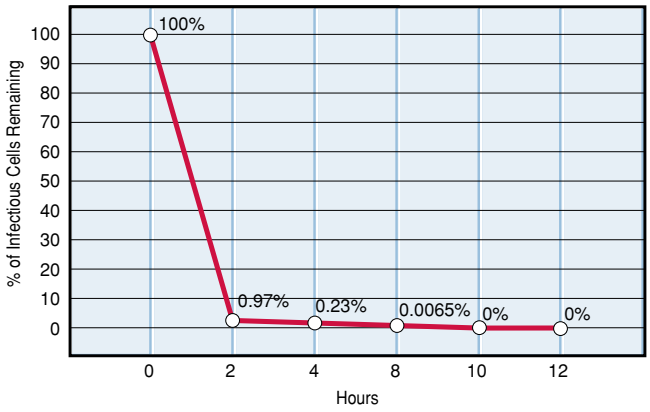
on reducing Avian Influenza A (H5N8) on **surfaces*** in 12-hour testing.

Testing by Kansas State University.

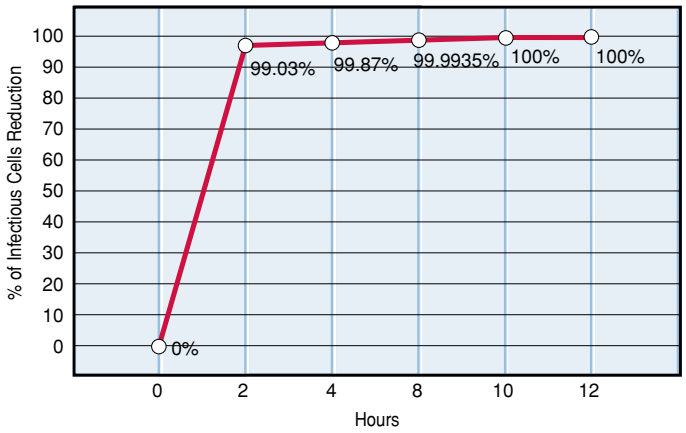
Avian Influenza A (H5N8) Inactivation with ActivePure (RCI)
Infectious Cells vs Time



Avian Influenza A (H5N8) Inactivation with ActivePure (RCI)
Percent of Infectious Cells Remaining vs Time



Avian Influenza A (H5N8) Inactivation with ActivePure (RCI)
Percent of Infectious Cells Reduced vs Time



*Scientific testing has demonstrated the use of EcoQuest's ActivePure technology to substantially reduce microbial populations on surfaces. Field results may vary based on environmental conditions. No claim with respect to airborne microbials is made based on these results. These results have not been evaluated by the FDA. This product is not a medical device intended to diagnose, treat, cure, or prevent any disease.

ActivePure (RCI) Inactivation of Avian Influenza

INTRODUCTION

The influenza virus, a member of the viral family Orthomyxoviridae, is characterized as being an enveloped single stranded negative sensed RNA virus (6) that can result in yearly endemic outbreaks and more severe world-wide pandemic outbreaks. Influenza A commonly infects human, swine, equine, and avian isolates. In the case of a pandemic outbreak, highly pathogenic avian influenza (H5N1) is currently the greatest threat due to current epidemic status in Asia, Europe, and Africa and continued threat for pandemic spread. Reassortment of genomic information of the influenza virus can result in a more pathogenic and infectious isolate is heightened during ongoing outbreaks, which could result in a devastating human-to-human transmissibility. Influenza virus is typically spread via aerosols, large droplets, or contact with infectious secretions or fomites (4).

Rapid containment of an outbreak is important for preventing further spread and minimizing the potential for reassortment to occur. Influenza has been shown to survive on nonporous surfaces for up to 48 hours and on material surfaces such as cloth, paper, or tissue for up to 12 hours after being deposited at approximately a 105 TCID50/ml level (1). In addition to surface sanitation and disinfection regimens, airborne inactivation of influenza virus is also vital to address predominant modes of transmission such as aerosol and large droplet (4). Environmental contamination with aerosolized droplets containing this pathogen can serve as a reservoir for infection and must be controlled by effective sanitation and disinfection protocols. Minimizing the degree of environmental contamination with highly effective decontamination measures would aid in the overall containment efforts of an outbreak.

The purpose of this study is to validate the complete inactivation of influenza A viruses using a low pathogenic avian influenza (H5N8) as a surrogate virus for the highly pathogenic avian influenza (H5N1) following exposure to the Radiant Catalytic Ionization-Cell™ (ActivePure-Cell™) system. The ActivePure-Cell™ system is an advanced oxidation tool which combines UV inactivation in the presence of hydroxical radicals so that synergy between two highly effective inactivation technologies occurs. Efficacy will be determined for dried inoculum on solid surfaces, in cell culture propagated inoculum, and nebulized in a controlled chamber. Efficacy will be determined by reduced or complete loss of infectivity in a cell culture system for treated samples compared to non-treated positive control samples.

MATERIALS AND METHODS

Virus and cells. Low pathogenic avian influenza H5N8 (H5N8, provided generously by the Centers for Disease Control and Prevention, Atlanta, GA) was propagated in 10 day embryonated hen eggs (Kansas State University Department of Poultry Science, Manhattan, KS) to approximately 107 log10 TCID50 (as determined in Madin Darby Canine Kidney, MDCK cells). Cells were maintained in Minimal Essential Medium with Earle's salts and L-glutamine (Invitrogen Corporation, Carlsbad, CA) and 2.2 g/L sodium

bicarbonate (Fisher Scientific, Hampton, NH) collectively referred to as MEM containing 10% fetal bovine serum (FBS, Hyclone Laboratories, Logan, UT) supplemented with antibiotics [2.5 mg/L amphotericin B; 0.67 g/L streptomycin; and 0.3 g/L penicillin G (all from Fisher Scientific)]. Infectivity media was made by adding MEM with the addition of 0.1% TPCK treated trypsin (Fisher Scientific) and supplemented with antibiotics (2.5 mg/L amphotericin B; 0.67 g/L streptomycin; and 0.3 g/L penicillin G).

H5N8 inactivation. Type 302 stainless steel (McMasterCarr, Altanta, GA) coupons (2 x 10 cm2, thickness 0.8 mm) were sterilized by autoclaving for 15 min at 121 C. In a biosafety class II cabinet, 100 µl of egg propagated H5N8 was added to each test coupon and spread to cover the entire surface using the pipette tip and allowed to dry completely for approximately 10-15 min. Then, the inoculated coupons were placed into a sterile transport container and transported to the test chamber. The test coupons were then attached to clips within the test chamber so that all sides of the coupon would be exposed to the ActivePure-Cell™ treatment. One coupon was removed prior to starting the ActivePure-Cell™ treatment to be used as the initial control sample. The ActivePure-Cell™ device was then turned on and samples were taken at various intervals (2, 4, 8, 12, 24 hours) by removing a test coupon and preparing it for virus recovery as described below.

Virus Recovery. H5N8 virus was recovered from the stainless steel surfaces by adding the test coupon to a sterile 50 ml conical vial (Fisher Scientific) containing 5 ml infectivity media. Tubes were then vortexed for 1 min. Endpoint dilution titration was conducted in MDCK cells by adding 220 µl from the 5 ml infectivity media containing any suspended virus to the first dilution well in a minimum of 6 wells of a 96 well microtiter plate containing confluent MDCK cells. Then, serial 1:10 dilutions were prepared by adding 20 µl from the first well into the next 6 wells each containing 180 µl infectivity media. The final well contained only 200 µl infectivity media to serve as a negative cellular control. Plates were incubated at 37 C, 5% CO2 for 48 hours. Cytopathic effect (CPE) was determined for each well and viral counts were reported as TCID50/ml as calculated by Reed and Muench (3).

Real-Time Reverse Transcription Polymerase Chain Reaction (rRT-PCR). Viral RNA was recovered using the QIAamp Viral RNA Mini Kit (Qiagen, Valencia, CA). Quantitative detection of the extracted influenza RNA was conducted using rRT-PCR using a fluorescently labeled TaqMan probe. The rRT-PCR primer and probe sequences were provided generously by the Molecular Genetics Influenza Branch, Centers for Disease Control and Prevention in Atlanta, GA. The detection threshold for successfully detecting influenza RNA was a FAM fluorescence signal ≥ 3 using the SmartCycler.

RESULTS

The average amount of H5N8 recovered from the stainless steel coupons in all experiments was 5.35 log10 TCID50/ml. Following treatment with the ActivePure-Cell™, the average log reductions of the H5N8 virus were 1.85, 2.79, 4.16, 5.35, and 5.35 log10 TCID50/ml following 2, 4, 8, 12, and 24 hour treatments (Figure 1) based on the recovery of infectious virus.

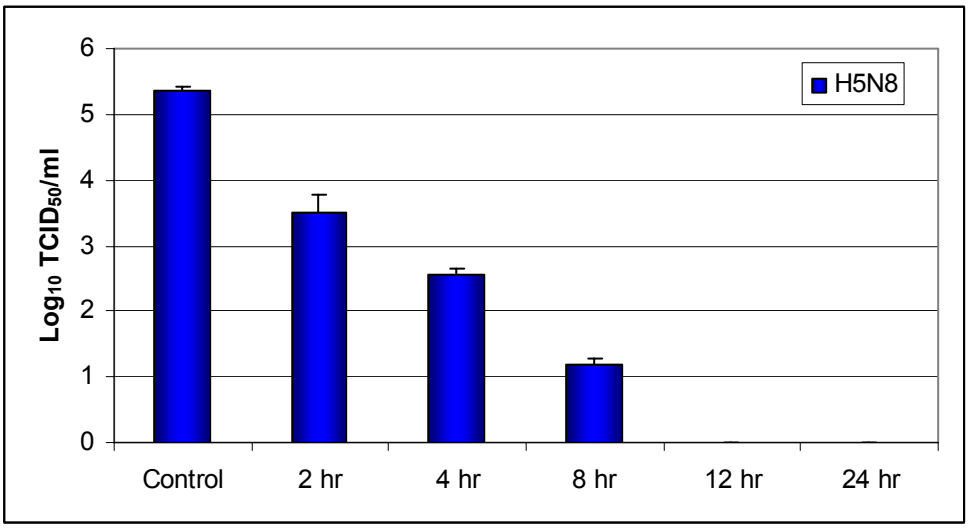


Figure 1: Recovery of H5N8 post-treatment with ActivePure-Cell™ based on $TCID_{50}$/ml in MDCK cells.

The average amount of viral H5N8 RNA recovered from the stainless steel coupons in all experiments was 4.00 log10 based on a quantitative RT-PCR available for influenza A viruses. Following treatment with the ActivePure-Cell™, the average log reductions of the H5N8 virus based on the amount of RNA recovered varied between 0.23 to 0.54 log10 following all exposure times (2, 4, 8, 12, and 24 hour) indicating that the mechanism of action for loss of infectivity was more likely due to disruption of the lipid envelope or structural proteins than with degradation of the viral nucleic acid (Figure 2).

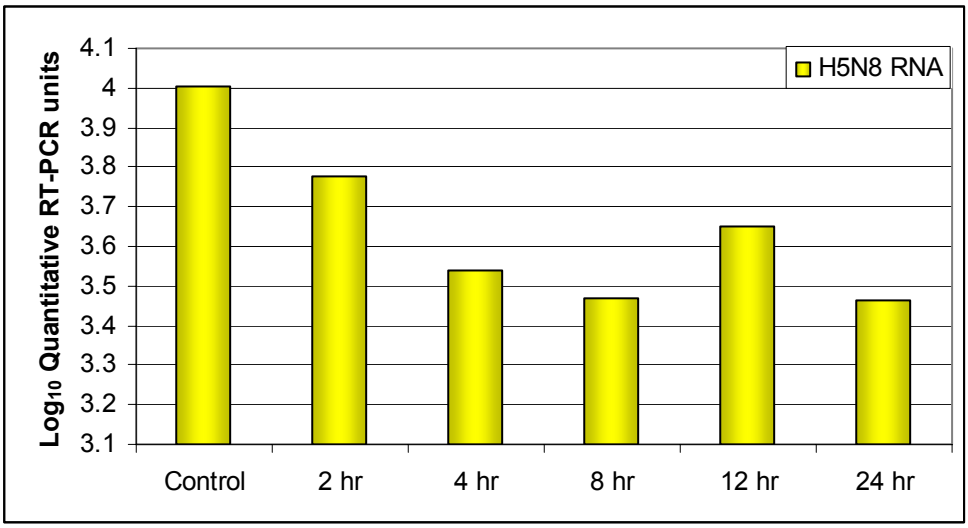


Figure 2: Recovery of H5N8 RNA post-treatment with ActivePure-Cell™ based on quantitative RT-PCR.

DISCUSSION

In an effort to better understand the inactivation of the influenza virus using the ActivePure-Cell™, the efficacy was evaluated using a low pathogenic avian influenza isolate, H5N8 inoculated onto stainless steel surfaces. Inactivation efficacy was determined following

the current EPA guidelines for determining virus disinfection (2) which allows the recovery of treated virus as endpoint dilution including a TCID50 recovery assay of infectious virus. In addition to the recovery of infectious virus, we wanted to determine if any disruption of viral RNA was occurring by using a quantitative RT-PCR assay specific for influenza A viruses in our experiments.

Based on the current EPA guidelines to achieve a > 4.0 log10 reduction in starting virus titer (2), ActivePure-Cell™ treatment for 8 hours or more resulted in the successful inactivation of the H5N8 isolate (Figure 1) for a starting contamination level of 5.35 log10 TCID50/ml. Additional testing would be required to determine if lower exposure times would result in complete inactivation for contamination levels lower than 5.35 log10 TCID50/ml, which might be more representative in a real outbreak (1, 5).

The quantitative RT-PCR results indicate that degradation of viral RNA (Figure 2) was not the major mechanism for viral inactivation, as the levels of RNA recovered after each treatment time were not significantly different from each other, $P > 0.05$. Other possible viral targets include the lipid envelope and structural proteins which were likely affected by the ActivePure-Cell™ treatment. The oxidative mechanism of this treatment likely disrupted the relatively susceptible envelope and could have resulted in denaturing the surface structural proteins of the influenza virus necessary for successful attachment and entry mechanism vital for infectivity.

The results obtained in this research experiment show that exposure to the ActivePure-Cell™ system for 8 hours results in the required level of inactivation of an avian influenza isolate, H5N8 which was used as a safe surrogate for the highly pathogenic H5N1 isolate. The mechanism of action of this technology is likely due to the oxidative chemistry resulting in both disruption of the lipid envelope and the denaturing effect on the structural viral proteins necessary for virus replication.

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Ongoing Research

Executive Summary by Allen Johnston - Chief Technology Officer, EcoQuest International

University of Cincinnati Test Results

EcoQuest ActivePure technology effect on air contaminants

Summary:
Testing of EcoQuest's Fresh Air Technology has been performed over an 18 month period at the Center for Health-Related Aerosol Studies in the Department of Environmental Health at the University of Cincinnati under the direction of Dr. Sergey Grinshpun, Professor.

Testing included two technologies used in the Fresh Air system; Negative Ionization and Photocatalysis (an innovative proprietary Photocatalytic Reactor called Radiant Catalytic Ionization - ActivePure).

Each technology was evaluated independently:
• Fresh Air Ionization technology was able to reduce airborne particles from indoor air by up to 250 times over natural decay (gravity)

• Fresh Air Radiant Catalytic Ionization (ActivePure -RCI) was able to inactivate approximately 90% of airborne microorganisms in less than 60 minutes. The microorganisms tested were MS2 Virus and B. Subtilis (used as a surrogate for Anthrax).

Dr. Grinshpun also concluded that the combination of the two technologies provided a much more significant reduction of airborne biocontaminants than either of the two technologies working independently.

About the Author:
Dr. Grinshpun is one of the most respected scientists in this important field of Aerosol Studies. Through his career, Dr. Grinshpun authored or co-authored about 390 scientific publications, including 120+ original articles in peer-reviewed journals, 90 book chapters and full proceeding papers, as well as about 180 conference abstracts. He has served as a reviewer, panel member or consultant to several federal agencies and professional associations nationally and internationally as well as for major companies and research institutions. He has also served on the Editorial Boards of four journals with international circulation. Dr. Grinshpun's accomplishments in aerosol research were recognized through the International Smoluchowski Award from the European Aerosol Assembly (1996, The Netherlands), the AIHA Outstanding Aerosol Paper Award (1997, USA), and the David L. Swift Memorial Award (2001, USA). He also received two John M. White Awards from AIHA (1997, 1998, USA) for his contribution to respiratory protection studies and Best Practice Award from the US Department of HUD (2000) for his studies of leaded particles in indoor air.

About the University:
University of Cincinnati is one of America's foremost Universities for Environmental Health.

About the Testing:
The testing by Dr. Grinshpun and his team focused on controlling aerosol contaminants in the indoor air through the application of two technology strategies:

1) Particle Concentration Reduction due to Unipolar Ion Emission
2) Microbial Inactivation due to the Photocatalytic reaction promoted by a Photocatalytic process called ActivePure - RCI (Radiant Catalytic Ionization)

The Results:
The paper concludes that the utilization of two mechanisms; ionization and oxidation, provide for significantly less exposure to potentially harmful contaminates in the air than either mechanism independently.

This conclusion is supported by showing ion induced air cleaning removes about 80% of viable airborne pathogens from a room air in 30 min, and the ActivePure-induced photoxidation inactivates about 90% of the remaining airborne microorganisms. The combination of both mechanisms resulted in an overall aerosol exposure reduction after 30 min by a factor of about 50, or an overall reduction/inactivation of approximately 98%.

The two active contaminants evaluated were:
1) B. subtilis bacteria
2) MS2 virions

Publication:
This research was peer reviewed and published in the journal of Environmental Science and Technology, January 2007, pages 606-612.

Note:
This testing was conducted in a controlled environment. Field results may vary based on environmental conditions. These results have not been evaluated by the FDA. This product is not a medical device intended to diagnose, treat, prevent, or cure any disease.

Environ. Sci. Technol. **2007**, *41*, 606−612

Control of Aerosol Contaminants in Indoor Air: Combining the Particle Concentration Reduction with Microbial Inactivation

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Center for Health-Related Aerosol Studies, Department of Environmental Health, University of Cincinnati, 3223 Eden Avenue, PO Box 670056, Cincinnati, Ohio 45267-0056

An indoor air purification technique, which combines unipolar ion emission and photocatalytic oxidation (promoted by a specially designed ActivePure-RCI cell), was investigated in two test chambers, 2.75m^3 and24.3 m^3, using nonbiological and biologica challenge aerosols. The reduction in particle concentration was measured sizes electively in real-time, and the Air Cleaning Factor and the Clean Air Delivery Rate (CADR) were determined. While testing with virions and bacteria, bioaerosol samples were collected and analyzed, and the microorganism survival rate was determined as a function of exposure time. We observed that the aerosol concentration decreased ˜ 10 to ˜100 times more rapidly when the purifier operated as compared to the natural decay. The data suggest that the tested portable unit operating in ˜25 m^3 non-ventilated room is capable to provide CADR-values more than twice as great than the conventional closed-loop HVAC system with a rating 8 filter. The particle removal occurred due to unipolar ion emission, while the inactivation of viable air borne microorganisms was associated with photocatalytic oxidation.Approximately 90% of initially viable MS2 viruses were inactivated resulting from 10 to 60 min exposure to the photocatalytic oxidation. Approximately 75% of viable B. subtilis spores were inactivated in 10min ,and about 90% or greater after 30 min. The biological and chemical mechanisms that led to the inactivation of stress-resistant air borne viruses and bacterial spores were reviewed.

Introduction

Exposure to respirable airborne particles and microbial agents may cause various health problems. Numerous techniques have been developed to reduce the exposure to indoor particles. Aerosol control in confined, poorly ventilated spaces, when the air exchange with filtration cannot be successfully applied, represents a particular challenge. Another challenge is to decrease the indoor concentration of specific airborne contaminants, e.g., viable biological particles. While some indoor air purification techniques aim solely at the aerosol concentration reduction, others are designed to inactivate viable bioaerosols (e.g., viruses, bacteria, and fungi).

Some commercial air cleaners generate excessive ozone (either as a primary biocidal agent or as a bi-product); these devices have raised public health concerns (*1*). Among various guidelines for ozone exposures, the following thresholds have been specified for occupational environments: 0.2 ppm for 2 h (*2*), 0.05−0.10 for 8 h (*2*), 0.1 ppm for 8 h (*3*), and 0.05 ppm for instantaneous (no time limit specified) exposure (*4*). For comparison, the outdoor air standard is 0.08 ppm for 8 h (*5*). Ozone generators can inactivate viable microorganisms; however, the inactivation occurs at concentrations significantly exceeding health standards (*6*, *7*).

Photooxidation involving UV radiation and TiO_2 as a photocatalyst has been applied for gas-phase detoxification of organic contaminants (*8*, *9*) and for inactivating microorganisms in water (*10*−*12*). Some effort has been made to explore its application for air cleaning inside a closed-loop system (*13*, *14*). The investigators reported significant photocatalytic inactivation of stress-resistant *Serratia marcesens* that occurred when aerosolized bacteria circulated in a closed-loop duct equipped with a TiO_2 filter for a relatively long period of time. Pal et al. (*15*) found similar effect for *Escherichia coli*, *Microbacterium* sp., and *Bacillus subtilis*; Keller et al. (*16*) reported considerable inactivation of airborne *E. coli* passing through a photoreactor coated with TiO_2 film. The biocidal effect of the photocatalytic oxidation can be attributed to photogenerated valence-band holes, hydroxyl radicals, hydrogen peroxide, and other reactive oxygen species. Lin and Li (*17*) tested the viability change in airborne bacteria and fungi exposed to photooxidation inside a small photoreactor for a very short time, on the order of a second. No significant decrease in the colony forming unit (CFU) count was observed during such a short time.

To our knowledge, no data are available on the effectiveness of portable UV/TiO_2-based air purifiers to inactivate viable airborne microorganisms in indoor air environments. These data are needed to assess the feasibility of photocatalytic oxidation for air purification in residential and occupational settings. Furthermore, for hybrid air purifiers, which involve several air cleaning mechanisms, no sufficient information is available to differentiate their particle removal efficiency and the biocidal capabilities, which both aim at reducing the bioaerosol exposure in indoor air.

In this study, we investigated a novel air purification technique that combines different aerosol/bioaerosol control mechanisms: unipolar ion emission and photocatalytic oxidation promoted by the "radiant catalytic ionization ActivePure - RCI" technique. Unipolar ion emission has been shown earlier to reduce the particle concentration in indoorair (18-20), but no scientific data are available on the efficiency of the hybrid-typetechnique.

Experimental Section

The indoor air purification process was investigated in the experimental facility shown in Figure 1. The particle removal was determined by measuring the concentration of challenge aerosols size-selectively in real-time. When testing with viable bioaerosols, the microorganism survival rate was also determined. The experimental protocols validated in our previous studies (*18*, *19*, *21*) were adopted. The experiments were conducted when a freestanding hybrid air purifier was

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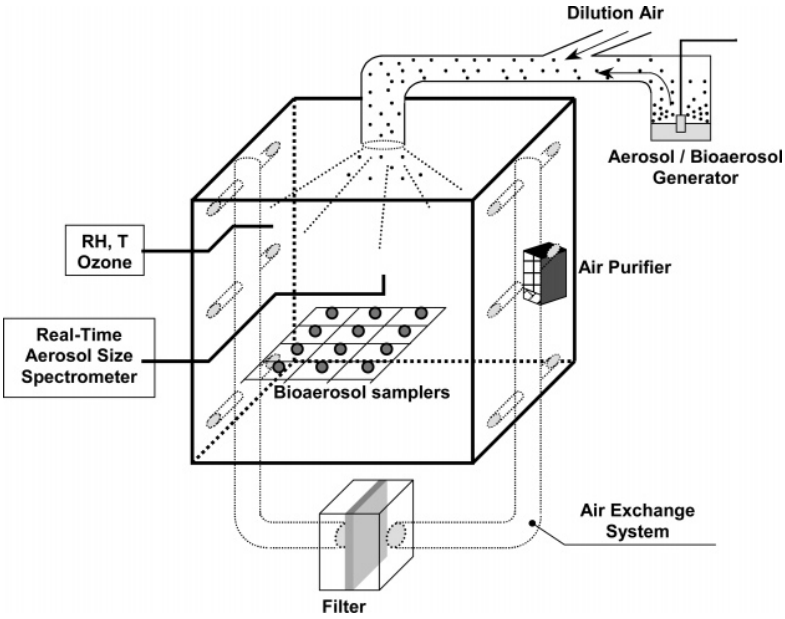

FIGURE 1. Experimental setup.

operating inside the chamber and when it was turned off. The challenge aerosol was generated from a liquid suspension using a Collison nebulizer (BGI Inc., Waltham, MA) and charge-equilibrated by passing through a 10-mCi Kr^{85} charge equilibrator (3M Company, St. Paul, MN). After being mixed with clean, HEPA-filtered air at a specific temperature (T = 24−26 °C) and relative humidity (RH = 21−30%), the aerosol entered the chamber. Following a 10−15-minute adjustment period established to achieve a uniform aerosol concentration pattern, the experiment began ($t = 0$).

In most of the tests, the aerosol concentration, C, and particle size distribution, $\Delta C/\Delta\log(d)$, were measured with an electrical low-pressure impactor (ELPI, TSI Inc./Dekati Ltd, St. Paul, MN), which utilizes the cascade impaction principle and also has a direct-reading capability to determine the concentration of particles of different aerodynamic sizes in 12 channels (each channel = impaction stage), from 0.041 to 8.4 μm (midpoint). When the experiments were conducted with viral aerosol that included particles smaller than the lower limit of the ELPI, we used a wide-range particle spectrometer (WPS; MSP Inc., Shoreview, MN). The WPS is a high-resolution real-time instrument combining differential mobility analysis, condensation particle counting, and laser light scattering to measure the diameter and number concentration of aerosol particles ranging from 10 nm to 10 μm.

For every measured particle size, d, the aerosol concentration at $t = 0$ was set to exceed the background level (obtained before the challenge aerosol was generated) by about 100-fold. First, the natural concentration decay was characterized by recording $C_{\text{natural}}(d, t)$ every 10 s with the ELPI and every 2.5 min with the WPS. Subsequently, the test aerosol was generated and mixed in the chamber again to reach the same initial concentration level. At $t = 0$, the air purifier was turned on and the concentration $C_{\text{AP}}(d, t)$ was monitored during and up to 120 min (or until the particle count decreased below the limit of detection). To quantify the efficiency of the particle removal exclusively due to the air purifier operation, the Air Cleaning Factor (ACF) was determined size-selectively as a function of time:

$$\text{ACF}(d, t) = \frac{C_{\text{natural}}(d, t)}{C_{\text{AP}}(d, t)} \tag{1}$$

In addition, the overall particle removal rate was calculated as

$$\lambda(d, t) = \frac{1}{t}\ln\left[\frac{C(d, t = 0)}{C(d, t)}\right], \tag{2}$$

and the particle removal rate (exclusively due to air purifier) was defined following the first-order kinetics as

$$\text{PRR}(d, t) = \frac{1}{t}\ln\left[\frac{C_{\text{AP}}(d, t = 0)}{C_{\text{AP}}(d, t)}\right] - \frac{1}{t}\ln\left[\frac{C_{\text{natural}}(d, t = 0)}{C_{\text{natural}}(d, t)}\right] \tag{3}$$

In case $C_{\text{AP}}(d, t = 0) = C_{\text{natural}}(d, t = 0)$,

$$\text{PRR}(d, t) = \frac{1}{t}\ln[\text{ACF}(d, t)] \tag{4}$$

This was needed to determine the Clean Air Delivery Rate (CADR), which, according to the ANSI/AHAM (American National Standards Institute/Association of Home Appliance Manufacturers) standard, is defined as

$$\text{CADR}(d, t) = V \times \text{PRR}(d, t)\ [\text{m}^3/\text{h}] \tag{5}$$

The CADR concept allows for comparison of air cleaning efficiencies of a freestanding air purifier and a closed- loop ventilation/air-filtration system in an air volume V (note that PRR is a function of V).

Two nonbiological challenge aerosols, NaCl and smoke, were used to study the particle removal by the air purifier. The generated particles were primarily in the size range of 0.02−2.0 μm, which includes ultrafine and fine fractions and represents most of the known viruses and bacteria. MS2 virus and *Bacillus subtilis* bacterial spores were the main biological challenge aerosols. Selected experiments were performed with *Pseudomonas fluorescens* bacteria.

MS2 bacteriophage, a 27 nm tailless non-enveloped icosahedral RNA-coliphage, relatively stable against environmental stress, has been used in the past as a simulant of most mammalian viruses, and it is known as an indicator for enteric viruses (*22*−*26*). Stock suspension of MS2 virus was prepared by adding 9 mL of Luria−Bertani broth to freeze-dried phage vial (ATCC 15597-B1). This suspension was

filtered using a membrane filter of 0.2 μm porosity and serially diluted so that the nebulizer suspension had 10^8-10^9 PFU/mL (PFU = plaque forming unit). MS2 phage titer was determined by following a modified plaque assay protocol of Adams (*27*); *Escherichia coli* (ATCC 15597, strain C3000) was used as the host organism.

B. subtilis is a gram-positive spore-forming bacterium with rod-shaped spores of approximately 0.7−0.8 μm in width and 1.5−1.8 μm in length (*28*). *B. subtilis* spores have previously been used in laboratory studies as a surrogate of environmentally resistant, pathogenic bacteria (*29*−*31*). Freeze-dried bacterial spores of *B. subtilis* (obtained from the U.S. Army Edgewood Laboratories, Aberdeen Proving Ground, Maryland) were activated at 55−60 °C for 25 min and then washed two times with sterile deionized water by vortexing followed by centrifugation at 7000 rpm for 7 min at room temperature. The total bacterial concentration in suspension was adjusted to 10^8-10^9 per mL using a hemacytometer. The viable bacteria were enumerated by cultivating on trypicase soy agar (TSA) media at 30 °C for 18 h; the viable (culturable) concentration in the nebulizer suspension was of the same order of magnitude as the total concentration, i.e., 10^8-10^9 CFU/mL (CFU = colony-forming unit). *P. fluorescens* bacteria (used in selected tests) are relatively sensitive to environmental stresses. Prior to aerosolization, vegetative cells of *P. fluorescens* (ATCC 13525) were cultured in trypticase soy broth at 28 °C for 18 h and washed similarly as *B. subitilis* spores.

When testing with biological particles, air samples were collected using Button Samplers (SKC Inc., Eighty Four, PA) equipped with gelatin filters (SKC Inc.) and operated at a flow rate of 4 L/min for 5 min. Eight Button Samplers were utilized in each test generating one blank, one background sample, three samples taken at $t = 0$, and the other three taken at a specific time interval; four time intervals were tested: $t = 10$, 15, 30, and 60 min. Additional selected experiments were performed by using a BioSampler (SKC Inc. Eighty Four, PA) to collect *P. fluorescens* and *B. subtilis*. The BioSampler efficiently collects viable bacteria (*29*) while the liquid medium minimizes the desiccation stress. As its cutoff size is too high to efficiently sample small MS2 virions, the BioSampler was not used as an alternative to gelatin filters for collecting MS2 virus.

The samples were analyzed for viable airborne virions (PFU) and bacteria (CFU) to quantify the percentages of those survived over time *t*. These were obtained with and without operating the air purifier. Our preliminary tests showed that the air purifier's operation considerably reduces the total bioaerosol concentration in the chamber due to ion emission. Therefore, the ion emitter was temporarily disabled in the hybrid unit when testing virus and bacteria inactivation to ensure sufficient number of microorganisms for determining the viable count at the end of the test.

An aliquot of 200 μL of dissolved gelatin filter extract was used for plaque assay to determine the number of airborne active (viable) virions (PFU/cm^3). Similarly, extract was cultivated on TSA plates to obtain the airborne concentration of viable bacteria (CFU/cm^3).

Additional testing was initiated to examine whether the biocidal effect of the air purifier took place indeed in the aerosol phase (and not after microorganisms were collected on filters). For this purpose, aerosolized microorganisms were collected on eight gelatin filters during 5 min in the chamber without air purifier. Four filters were analyzed for viable microorganisms immediately after this test, while the other four were exposed to the air purifier in the chamber for 10, 15, 30, and 60 min and then analyzed. The comparison of two sets allowed examining if the microorganism inactivation occurred on filters during the collection process.

The ozone level and the air ion concentration were monitored in real-time in the chamber using an ozone monitor (PCI Ozone & Control Systems, Inc., West Caldwell, NJ) and an air ion counter (AlphaLab Inc., Salt Lake City, UT), respectively. The air temperature in the test chamber was 24 ± 2^0C and the relative humidity ranged from $22 \pm 2\%$ to $28 \pm 2\%$ as monitored with a thermo/hygrometer pen (Fischer Scientific Co., Pittsburgh, PA).

The purifier prototype (Ecoquest International Inc., Greeneville, TN) used in the study utilized an ion emitter and a specially designed ActivePure cell. The former produces negative ions in to indoor air, where they are acquired by aerosol particles. It is important to note that this method is different from air cleaning by charging particles at the entrance of the purifier and subsequently collecting them on metal electrodes by electrostatic precipitation. The ActivePure cell features a flow optimized target structure comprising matrices of elongated tubular elements made of polycar-bonate and arranged in a parallel orientation on opposite sides or alternatively on four sides of a broad-spectrum UV light source. The UV lamp utilizes argon gas with mercury and carbide filaments with a spectral output between 100 and 367 nm. Besides, a coating was applied to the target structure of the cell comprising hydrophilic properties and containing the following grouping of materials: titanium dioxide, rhodium, silver, and copper. As a result, a photo-catalyticoxidation forms reactive species, such as hydroxyl radicals, valence-band holes, superoxideions, and hydrogen peroxides.

The tests were conducted in two indoor test chambers, including a large walk-in chamber (24.3 m^3) that simulated a residential room and a smaller chamber (2.75 m^3) that simulated a confined space (e.g., bathroom, small office area, or automobile cabin). The particle removal was investigated in both chambers, whereas the bioaerosol viability tests were performed in the smaller chamber that was made of stainless steel and allowed bio-decontamination. The air purifier was tested in non-ventilated chambers (no air exchange) as it is known that portable air cleaners are primarily beneficial in poorly ventilated spaces (*20, 21*). Air exchange was introduced only when testing the closed-loop ventilation/air-filtration system equipped with an HVAC filter to compare its performance to that of the portable air purifier in terms of CADR. The ventilation/air-filtration system was also deployed to clean the test chamber between experiments. In most of the tests, the air purifier operated in the corner of the chamber, facing the center. A separate experiment was carried out to examine whether its location and orientation affected the ACF.

Results and Discussion

Particle Removal from Air. Figure 2 shows the evolution of the concentration and particle size distribution of NaCl aerosol when the air purifier operated in the large test chamber. As seen from this example, the aerosol concentration of 0.1 μm particles decreased by a factor of 28 in 1 h and by a factor of about 250 in 2 h; the corresponding decreases for 1 μm particles were approximately 10- and 50-fold. When testing with smoke particles, the aerosol concentration decreased even more rapidly. The above levels of the aerosol concentration reduction are considerably greater than those predicted by either tranquil or stirred natural decay models (*32*). This result was obtained when both the air ion emitter and the RCI cell operated in the unit. Interestingly, statistically the same particle reduction effect ($p > 0.05$) was observed when the RCI cell was turned off and only the ion emitter operated. The latter finding provides the evidence that the particle removal was achieved as a result of unipolar ion emission but not due to photocatalytic reactions.

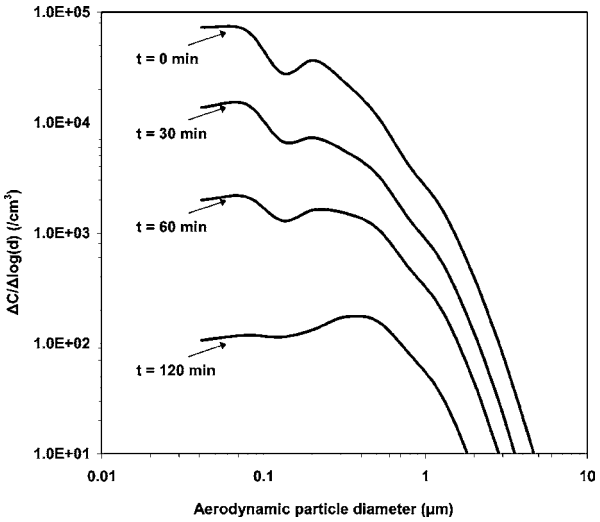

FIGURE 2. Particle concentration and size distribution of NaCl aerosol as measured with the ELPI in the 24.3 m³ chamber with the air purifier operating facing the chamber's center at 1.7 m from the measurement point. No ventilation in the chamber. The initial total aerosol concentration = 1.50 × 10⁵ /cm³.

This finding agrees with previously published data on the effect of unipolar air ionization on the airborne concentration (*18*−*21*). The air purification is particularly efficient at higher initial aerosol concentrations (>10^4 particles/cm^3) that ensure adequate interaction between the air ions and aerosol particles. As mentioned above, the effect is expected to be much more pronounced in non-ventilated environments than in ventilated ones.

The aerosol reduction was especially high for the particles of $d \leq 0.3$ μm. E.g., when the air purifier with an ion output of ~10^{12} e/sec continuously operated in a corner of the 24.3-m^3 chamber facing the center for 2 h, ACF reached ~30−70 for $d = 0.08-0.3$ μm and ~13−16 for $d = 0.8-2$ μm (in the tests conducted with NaCl and smoke as challenge aerosols). The same ACF levels may be achieved more rapidly in indoor environments of smaller volumes and slower in larger spaces. The experimental trends agree with the ion-induced aerosol removal model (*20*).

The ACF was found to depend not only on the operation time and the particle size but also on the location/orientation of the purifier in the chamber. For example, a corner location facing the center of the room was found preferable as opposite to the orientation facing the wall. The difference in ACF obtained for the center and corner locations was significant and increased with the operation time. The shaded area in Figure 3 presents the ion-induced Air Cleaning Factor when the particle size-selective data were integrated over the measured sizes of NaCl particle up to 2.5 μm and averaged over the three selected locations/orientations in the 24.3-m^3 chamber: in the corner facing the center, in the center, and at 80 cm from the wall facing it.

Figure 4 presents the CADR values achieved by operating the tested air purifier for five selected sizes of NaCl and smoke particles acting as aerosol contaminants in the non-ventilated 24.3 m^3 chamber. The CADR ranges approximately from 42.1 ± 0.1 to 62.1 ± 1.8 m^3/h for NaCl particles of $d = 0.04-1.99$ μm, and from 72.4 ± 0.9 to 115.5 ± 10.8 m^3/h for smoke particles of the same size range. The difference may be attributed to different ability of NaCl and smoke particles to acquire electric charges from air ions, which results in their different mobilities and subsequently different migration velocities. The above explanation seems valid given that unipolar ion emission was shown to be the major mechanism causing the aerosol particle concentration reduction.

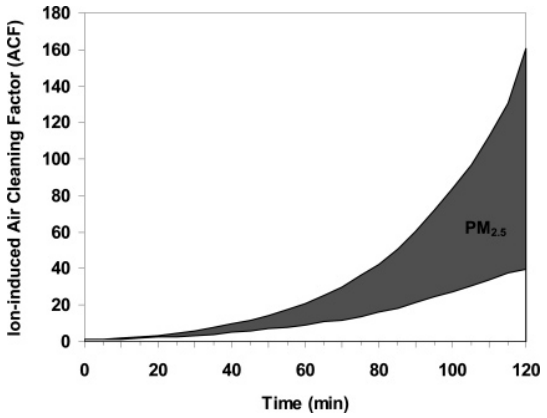

FIGURE 3. The ion-induced Air Cleaning Factor (ACF) for $PM_{2.5}$ NaCl as measured with the ELPI and integrated for different locations and orientations of the air purifier in the 24.3 m³ chamber. No ventilation in the chamber. The initial $PM_{2.5}$ aerosol concentration = (0.356−1.50) × 10⁵/cm³.

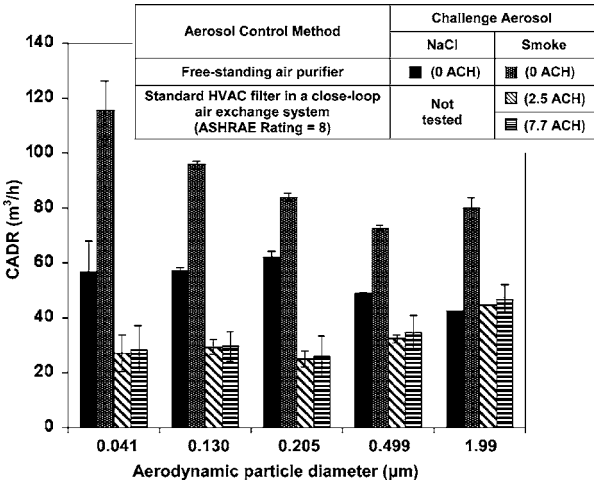

FIGURE 4. Clean Air Delivery Rate (CADR) determined for the NaCl and smoke aerosols as measured with the ELPI in the non-ventilated 24.3 m³ chamber. The performance of the air purifier is compared to that of a standard HVAC filter (ASHRAE rating = 8) installed in the closed-loop air exchange system of the chamber.

In addition, Figure 4 presents the CADR values achieved by the closed-loop air exchange system equipped with a standard ASHRAE rating 8 HVAC filter at two air exchange rates, 2.5 and 7.7 ACH. The data suggest that the tested portable air purifier operating in about 25 m^3 non-ventilated room is capable to provide a CADR more than twice greater than the conventional central HVAC system with the rating 8 filter. Obviously, more efficient particulate filters provide more rapid reduction of aerosol contaminants and may perform better than the tested air purifier. For example, compared to the portable unit, HEPA filter installed in the closed-loop air exchange system of the 24.3 m^3 chamber provided approximately 4- and 3-fold greater CADRs at 2.5 and 7.7 ACH, respectively, when challenged with NaCl particles, and 2.2- and 1.4-fold greater when challenged with smoke particles. However, HEPA filters are rarely used in residential central HVAC systems because of the high-pressure drop and the loading effect on their performance.

The particle removal from indoor air by the hybrid air purification technique was also investigated in the smaller (2.75 m^3) chamber, which otherwise was utilized primarily for assessing the viable microorganism inactivation. The CADR values obtained with MS2 virions from the WPS measurements were 73 ± 5 m^3/h, which is in the CADR-

TABLE 1. Percentage of Airborne Microorganisms Survived over Time *t* in the 2.75 m³ Chamber with the RCI-cell Operating in it, as Measured via PFU Count (for MS2 Virus) or CFU Count (for *Bacillus subtilis* Endospores)[a]

exposure time, t (min)	percentage (mean ± SD) of airborne microorganisms survived in the chamber with air purifier operating during time *t*	
	MS2 virus, $[PFU/cm^3]_t/[PFU/cm^3]_{t=0}$	*Bacillus subtilis* endospores, $[CFU/cm^3]_t/[CFU/cm^3]_{t=0}$
10	9.3 ± 2.0 (n = 5)	24.1 ± 3.7 (n = 2)
15	9.2 ± 4.3 (n = 12)	15.7 ± 1.7 (n = 3)
30	8.3 ± 1.1 (n = 8)	7.9 ± 1.1 (n = 3)
60	10.3 ± 1.7 (n = 5)	10.1 ± 1.3 (n = 3)

[a] Bioaerosol sampling was conducted with the Button Sampler equipped with gelatin filters. n = number of replicates.

range obtained for NaCl and smoke particles in the large chamber for the viral sizes. This suggests the feasibility of using nonbiological particles to determine the ion-induced aerosol reduction of bio-particles of the same size range. Furthermore, this finding implies that, at least for the particle size range representing MS2 virions, PRR due to ion emission in indoor air environment is inversely proportional to the air volume [see eq 5].

Ozone. In both test chambers (non-ventilated), the ozone concentration gradually increased as the purifier was continuously operating. In the 24.3-m^3 chamber, it increased from 0.006 to 0.05 ppm in about 35 min, while in a smaller (2.75-m^3) chamber the same increase occurred in approximately 5 min. However, once an air exchange was introduced (as low as 1 ACH), the ozone concentration in the 24.3-m^3 chamber did not significantly increase as compared to the initial level ($p > 0.05$). Our monitoring data obtained with the tested unit operating in a non-ventilated room of ~100 m^3 (not presented here) suggest that the ozone level can be kept below 0.05 ppm while the unit continuously operates for many hours.

Some air purifiers utilizing ion emission and, to a greater extent, the photocatalytic oxidation may cause greater increase of indoor ozone concentration than the tested one. The use of such devices in confined occupied air spaces may not be appropriate as their continuous operation may eventually lead to excessive ozone levels and, in the presence of certain chemical compounds, produce nanoparticles (*33*). Although the unipolar ion emission has a potential to suppress this effect, it seems important to keep the ozone level below existing thresholds. We believe that the solution can be found by implementing an intermittent regime (as an alternative to continuous one), which allows the air purifier operating until the ozone reaches a certain level, after which the ozone-generating element is automatically turned off to allow the ozone concentration to drop; then the cycle can be repeated.

Microbial Inactivation. Table 1 summarizes the microbial inactivation results. Only approximately 10% of initially viable MS2 virions survived 10−60 min exposure to the purifier in the chamber and about 90% were inactivated. When the natural concentration decay of aerosolized MS2 was monitored in the chamber (with no purifier operating), we found that the concentration of active viruses was relatively stable: the decrease did not exceed 20.3 ± 0.9% during 1 h. The data suggest that the viral inactivation occurs rather quickly since the percent of survived virions did not show dependence on the exposure time for t = 10−60 min. Thus, a relatively short time may be sufficient to reduce the percent of viable viruses in an air volume by a factor of 10 while those that survived showed remarkable resistance to the continuing stress. When aerosolized virions are exposed to photocatalytic oxidation, the hydroxyl radicals can affect the protein capsid and binding sites, thus disabling the virus's subsequent interaction with the host and formation of PFUs (*34*). Additionally, the TiO_2 photocatalytic cell may produce oxidative damage to the virus capsid (*35*) and the radicals may cause alteration in the virus's genetic material (*36*, *37*). Our findings suggest that the hybrid air purifier may be used continuously for short time intervals or in intermittent regime to achieve considerable virus inactivation rate. On the other hand, a prolonged operation of the air purifier is believed to be advantageous in environments with a continuous supply of "fresh" active virions.

Approximately 75% of airborne *B. subtilis* spores exposed to the air purifier were inactivated during the first 10 min, 85% during the first 15 min, and about 90% or greater after 30 min (Table 1). Between 30 and 60 min of exposure, we did not observe significant decrease in the number of survived spores (similar to the trend found for virions), which suggests a nonlinearity of the effect. The natural decay in the culturable count was not significant ($p > 0.05$) during 1 h, as measured using the Button Samplers equipped with gelatin filters. However, the overall standard deviation of the data obtained in these control tests was as high as 58% and the CFU counts from filters were close to the detection limit. To address this issue, we measured the natural decay of viable *B. subtilis* spores with the BioSampler at $t = 0$ and at $t = 2$ h. It was confirmed that the viability was constant within about ±20% in the absence of the air purifier.

In bacteria, the inactivation process by reactive hydroxyl radicals can proceed in five reaction pathways:

- oxidation of coenzyme A causing inhibition of cell respiration and cell death (*38*);
- destruction of the outer membrane of bacterial cells (*12*);
- oxidation of unsaturated phospholipid in bacterial cell membrane (*39*);
- leakage of intracellular K^+ ions (*11*); and
- detrimental effects on DNA and RNA (*36*, *37*).

One reason that the inactivation of *B. subtilis* endospores was time-dependent is their thick membrane layer containing peptidoglycans. This is consistent with the study of Matsunaga et al. (*40*), who found that photooxidation of coenzyme A by the TiO_2 photocatalyst was not entirely effective against the algae *Chlorella vulgaris* in water because of its thicker cell wall. Some other self-defense mechanisms of bacteria against the oxidation stress, including synthesis of superoxide dismutase enzymes, can also slow down the inactivation process (*41*).

Although the time was a factor in the bacterial spore inactivation, the viability loss occurred relatively quickly for both the MS2 virus and *B. subtilis*. This can be attributed to rapid interaction of valence-band holes (h^+) ($TiO_2 + h\nu \rightarrow h^+ + e^-$.) with the organic substances, which are present in the viral and bacterial outer walls or membranes. The above-mentioned interaction likely occurs before considerable number of hydroxyl radicals (·OH) is generated in the air volume. Although previous studies (*11*, *12*) emphasized the role of hydroxyl radicals ($H_2O + h^+ \rightarrow {}^{\cdot}OH + H^+$), these radicals may not be the primary factor in microbial inactivation, particularly in the air. Furthermore, since our experiments were conducted in relatively dry air (RH < 30%), water molecules were not predominant species in contact with the catalyst, and thus the contribution of hydroxyl radicals was likely much lower than in liquids. Shang et al. (*9*) have concluded that in the gas phase, organic compounds, such as heptane, can readily interact with photogenerated holes while the interaction with water vapor molecules is not as prominent. Alberici and Jardim (*8*) have reported that the valence-band holes generated from TiO_2 photooxidation are capable of oxidizing any organic compound. The process also produces hydrogen peroxide ($O_2 + e^- \rightarrow O_2^{\bullet -}$; $O_2^{\bullet -} + H^+ \rightarrow HO_2^{\bullet}$; $2HO_2^{\bullet} \rightarrow O_2 + H_2O_2$), which can freely penetrate into cell membranes and walls and cause microbial inactivation

(*42*). Further biochemical studies on the role of gas-phase TiO_2 oxidation on the airborne microorganisms as well as studies on the reaction kinetics at the aerosol phase seem worthwhile to further examine the above interpretations.

Experiments with *P. fluorescens* revealed CFU counts below the detection limit both in the test and control samples. In contrast to *B. subtilis* endospores, even a very short exposure to ambient air (RH < 30%) considerably decreased the viability of aerosolized *P. fluorescens* vegetative cells, which are known to be stress-sensitive. Perhaps, microorganisms sensitive to desiccation stress are more usable for this kind of test if the test is performed at higher relative humidity levels.

Additional control experiments were performed to investigate if the viability decrease found for MS2 virus and *B. subtilis* spores occurred in the aerosol phase or on the sampling filter. For MS2, we found that 1835 ± 270 PFU/mL and 1855 ± 325 PFU/mL developed when filter extracts were cultivated from unexposed and 10-min exposed gelatin filters, respectively. For *B. subtilis*, we observed 1770 ± 275 CFU/mL and 1125 ± 410 CFU/mL in extracts taken from unexposed and 60-min exposed filters, respectively. No significant changes in either viral or bacterial viability occurred as a result of a non-aerosol exposure ($p > 0.05$). Thus, these findings confirm that the viral and bacterial inactivation observed in our tests indeed occurred in the aerosol phase and was not associated with the inactivation on filters.

Combined Effect (Sample Calculation). It was concluded that the particle removal took place solely due to unipolar ion emission, while the inactivation of viable airborne MS2 virions and *B. subtilis* spores occurred due to the photocatalytic reaction promoted by the RCI cell. Both mechanisms working simultaneously in a hybrid type air purifier may result in considerable decrease of the exposure to pre-existing viable aerosol biocontaminants in indoor environment. Ozone produced by the RCI cell is not believed to cause significant microbial inactivation because its level was not sufficient. Tseng and Li (*43*) referred to 3.43 ppm as an appropriate level for airborne MS2 virus, and Li and Wang (*44*) did not observe any inactivation of airborne *B. subtilis* spores at O_3 as high as 20 ppm.

The following estimate was made based on the experimental data obtained in this study. Assuming that the ion induced air cleaning removes about 80% of viable airborne pathogens from a room air in 30 min and the ActivePure (RCI) -induced photoxidation leaves only 10% of the remaining airborne micro organisms viable, the overall aerosol exposure to the viable pathogen in this room after 30 min is reduced by a factor of about 50.

The observed rapid inactivation of micro organisms makes unnecessary to run the ActivePure cell continuously. The data suggest that it can be used "part-time" for 10-30 min and "rest" for about 1-2 h until the background ozone level is reached (proposed above as an intermittent regime), while the ion emission can take place continuously to keep the aerosol concentration decreasing.

Acknowledgments

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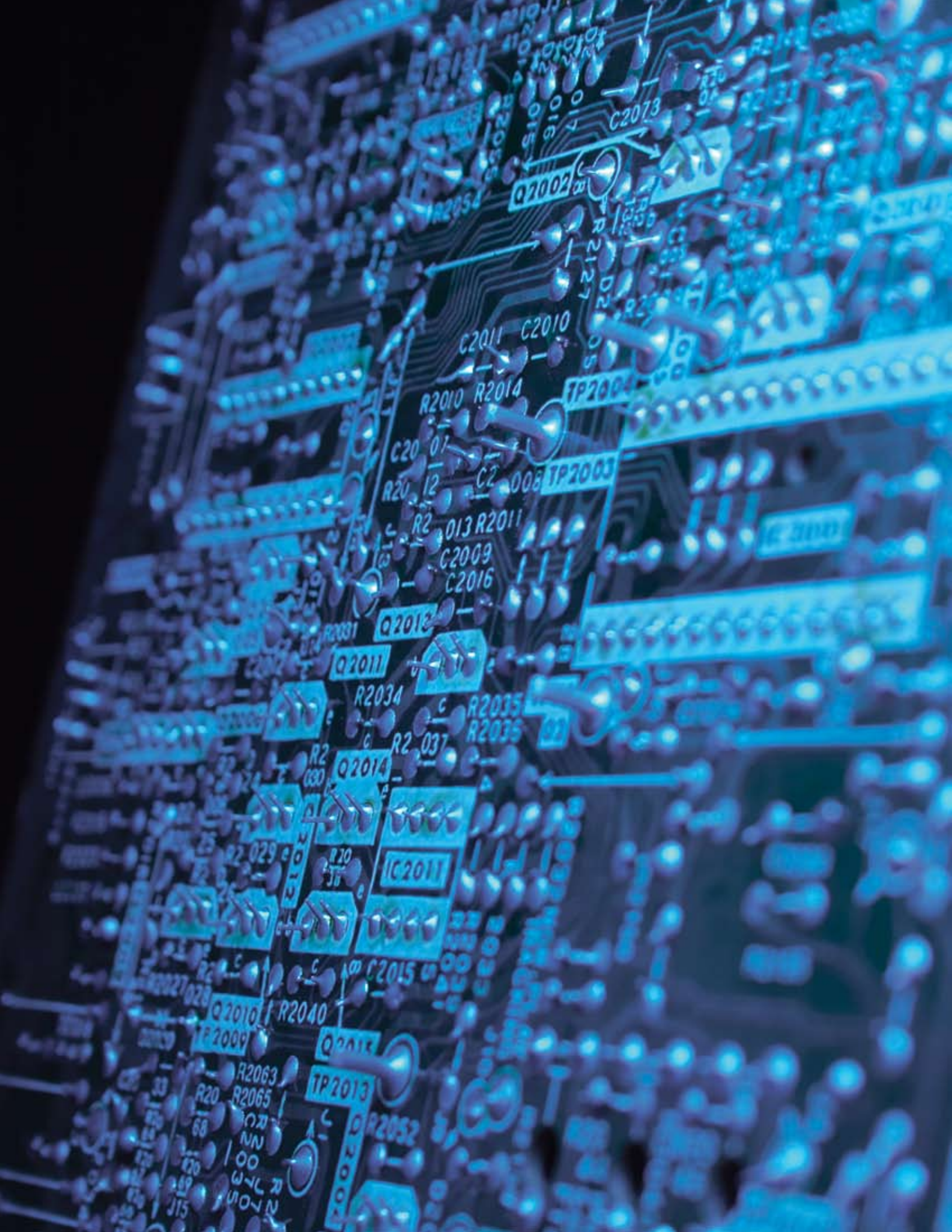
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User Experiences

WALNUT TREE
DRYCLEANERS LTD

21st August 2006

Re: Fresh Air for Fire Restoration

To whom it may concern

CRDN Walnut Tree are part of the Certified Restoration Dry Cleaning Network (CRDN), based in Milton Keynes, Buckinghamshire.

We work closely with National and Independent Contractors, Insurance Companies and Loss Adjusters on Insurance Claims which include items such as clothing, curtains, bedding, soft toys, shoes and handbags. Items contaminated by smoke, water, builders dust, oil etc can be restored saving the Insurance Company up to 84% of the replacement cost.

A strategic part of the technology used to restore textiles is a product introduced to us by Claire which uses 'Fresh Air' as part of the process.

When we were initially given a unit to trial we really put it through its paces. We tried it with every type of fabric we could find - even really difficult things which you have no other means of treating - like pillows, heavy duffel coats and delicate costumes. We also tried it out on as many different types of smoke damage as we could find. Whatever we tried, it didn't let us down, furthermore, our girls were amazed at just how quickly Claire's equipment eradicated the smoke from the textiles!
I've willingly recommended it to all the other members of CRDN and other related businesses, it simply is a great product!

Claire also tells me she will soon have a product which will enable us to wash clothing without detergent. I can't wait to see that!!!

Thanks again

John Cushing
Managing Director
CRDN Walnut Tree

The GRATZ National Bank

April 8, 2006

Re: Testimonial for the Ecoquest Fresh Air Machine

Six months ago we purchased a Fresh Air Machine from Jim and Jackie Sleter and have been extremely pleased with its performance. As a heavy smoker, I am always concerned with the air quality in our offices and this fresh air machine has had remarkable results. The air quality has been greatly enhanced and the "away" feature does a great job!

I would strongly recommend this machine to anyone interested in improving the air quality in their environment. Its performance has been excellent with little maintenance and its quiet operation.

Many thanks Jim and Jackie!

Theodore R Bonwit, Jr.
President

HUMAN SERVICES ASSOCIATES, INC
ADMINISTRATION

June 20, 2006

Dear Mr. Bruce Robertson:

On behalf of Human Services Associates, Inc., we would like to thank Fresh Air Systems for the air machines. We were surprised and pleased after using your air machines.

Recently the CBC of Seminole had a complete new roof system installed and the smell during the process was horrible. Thanks to Bruce who recommended we use the machines from Eco Quest International for removal and cleaning of the air in the building. We agreed and he promptly brought seven machines in immediately and placed throughout our facility which is 22,000 square feet. When I first saw the machines, I laughed to myself and thought how in the world these little machines could work in such a large space. The machines not only took out the foul order they cleaned the air too. The building smelled fresh and clean.

Thanks to Fresh Air Systems, the employees were able to remain in the building during this week during the installation of the new roof. Human Services Associates, Inc. will use the machines again in the future when any other projects arise.

Thanks again for the prompt courteous service you provided for CBC of Seminole.

Sincerely,

S. Ciccone

Sherry Ciccone
Facilities Manager

Dear John:

What a difference the Fresh Air by EcoQuest has made!

We have a lounge/bar that overlooks the playing field that used to get extremely smoky. Now, even on the busiest night it doesn't get smoky. The bartenders, as well as the customers, leave without smelling. They love it!

The whole building smells better.

I recommend it to every tavern, restaurant, and office.

Thanks for such a great product.

Jeanne Herries
Jamestown Sports Complex
Office Manager
10/10/2006

VILLA VENTURA HAIRSTYLES

Hi Nola,

Just wanted to let you know, I have a Beauty Salon in a Retirement Center. Of course we do lots of permanents and sculptured nails, both leave the shop stinky. You introduced me to Fresh Air. I had the unit for three days and when we returned to work it was unbelievable how clean the shop smelled! The clients love the way the shop smells and how fresh every thing is. We love our unit so much we got three more for our home. Thank you Nola for introducing the Fresh Air to me!

Thanks,

Linda
Linda Vest, Owner

June 22, 2006

Mike,

My father had left a skillet with sausage cooking on the stove and forgot about it. It burned and filled our outside kitchen wilth smoke and an aweful smell. The fresh air purifier removed the burnt odor and gave the room a fresh smell.

After 25 years of cooking in that outdoor kitchen, I was surprised how the fresh air purifier not only removed the burnt odor, it also removed the old stale odor in the room.

Thanks,

Shelley Delapasse

BST
Brown Sugar Café

April 28, 2007

Re: Testimonial for EcoQuest Internationals' Space Certified Technology

My husband and I have been in the Thai restaurant business for over 10 years. Because we've been in and out of Thai kitchens for so long, our noses have become a little desensitized, do to the many spices used in Thai cooking along with various other restaurant odors.

So, in September of 2005 when Joseph and Catherine Kappel first approached us about integrating air purification into the restaurant, we were a little skeptical to say the least. First we incorporated the Fresh Air stand alone unit in our Cambridge restaurant. It made the air feel and smell fresh. However, Joseph felt we could do better. This restaurant has tall ceilings and several thousand square feet, so he recommended installing a DuctwoRx 14 inch commercial probe into the HVAC system.

The results were amazing. Within the first few hours, we began to notice a distinctive, natural odor throughout the restaurant. The Fresh Air unit was like being in 2nd or 3rd gear but adding the DuctwoRx was like putting it into overdrive. Most strikingly was down a small corridor that leads to the restrooms where the air was very dead and stagnant, it was transformed and the freshness permeated the entire area. We also notice that even though Thai cooking odors are continually being introduced, they now have the aroma of a freshly cooked meal. There are no lingering odors as in the past.

Given our positive experience in Cambridge, we soon integrated another DuctwoRx commercial probe and a Fresh Air into one of our restaurants in Boston. In addition, we only needed one Fresh Air in our 2nd restaurant in Boston because it has much less square footage. Furthermore, we've had a DuctwoRx residential probe installed in our home along with the Fresh Air, a Spectrum for our sons room and a Living Water purifier, all from EcoQuest International.

Thanks to EcoQuest and the Kappel's, our family along with our many hundreds of loyal customers, all enjoy a healthier and safer environment. We can wholeheartedly recommend EcoQuest products to anyone who wishes to improve their indoor air and/or water quality, at work and at home!

With Gratitude,

Tu and Danee Pinyochon
Owners and Operators
Brown Sugar Café


800
600
400

EcoQuest International

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