Confidential Research Report

Fungicidal Efficacy of SCO Technologies' Ozone Generation System Against *Aspergillus versicolor*

For

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Purpose

To determine the fungicidal efficacy of Medallion's proprietary ozone generation system against viable *Aspergillus versicolor* and associated mycotoxins.

Materials

1. *Aspergillus versicolor* (NRRL 238)
2. Potato Dextrose Agar (PDA), Kalmar Laboratories
3. Butterfield's Buffer (BFB), Kalmar Laboratories
4. Methanol
5. Test Equipment
    a. ARTI HHPC6 Particle Counter
    b. Ozone meter, provided by Medallion Healthy Homes
    c. SCO Technologies' Ozone Generator System, provided by Medallion Healthy Homes
    d. Humidifier - specifications
6. Dry wall sections, 3 x 1.5 x 0.5"
7. Incubator, 25°C
8. Microbial Test Chamber: Custom built chamber (11 x 9.5 x 8 ') constructed from non-porous, non-reactive construction materials and equipped with negative displacement ventilation and HEPA filtration.

Methods

The challenge organism was cultivated on Potato Dextrose Agar (PDA) at 25°C until confluent growth was obtained (Figure 1). Subsequently, the organism was harvested with a moistened sterile swab and inoculated on to the drywall test section. The inoculated drywall was placed into a sealable plastic bin on top of moistened paper towels and incubated at room temperature until confluent growth was observed (Figures 2 and 3).

On the day of the experiment, the microbial test chamber was placed under negative pressure and purged with HEPA filtered air to ensure no residual particulates were present. The inoculated drywall was positioned vertically inside the chamber with the side containing fungal growth facing outward. Representative 100 cm^2 areas of the drywall were sampled for viable fungi and mycotoxins using a sterile swab pre-moistened with Butterfield's Buffer and methanol, respectively (Figures 4 and 5). Medallion Healthy Homes personnel treated the chamber with ozone for 24 hours. Test chamber conditions were monitored for particulates, humidity, and temperature during the 24 hour treatment interval using ARTI HHPC6 Particle Counter. Once the ozone application was complete and safe ozone concentrations within the chamber were confirmed, the chamber was opened and representative 100 cm^2 areas of the drywall section were sampled in the same manner as the "Pre-Ozone" samples.

All samples were tested for viable fungi and mycotoxins. Swabs that were tested for fungal growth were cultured via the Spiral Biotech Autoplater and incubated for 7 days at 25°C. All analytical procedures met criteria set forth in Aerotech's Standard Operating Procedure for the Preparation of Swab, Bulk, CarpetChek, and Water Samples for Viable Fungal Analysis, 20-0096.00. Mycotoxin surface samples were analyzed via High Performance Liquid Chromatography (HPLC) with Diode Array Detector (DAD) and Fluorescence Detector (FLD). All analytical procedures met criteria set forth in Aerotech's Standard Operating Procedure for the Determination of Mycotoxins using HPLC with DAD and FLD Detectors, 05-012.00.

Results and Discussion

The results of the test chamber conditions during treatment are shown in the graphs in Appendix A. The average relative humidity was 56%; average temperature was 92° Fahrenheit. Four ozone measurements were collected over the 24 hour period, the average ozone concentration was 14.65 parts per million (ppm).

As shown in Table I, the inoculated drywall section contained 3,400 viable *Aspergillus versicolor* per square centimeter prior to ozone treatment. Following ozone treatment, the *Aspergillus versicolor* was non-detectable at a detection limit of 18 colony forming units per square centimeter, indicating a 190 fold reduction (Figure 6).

The results for the mycotoxin analysis are also shown in Table 1. The *Aspergillus versicolor* drywall boards did not test positive for the presence of mycotoxins in the pre-ozone samples therefore post ozone concentrations could not be evaluated.

Wendy Aber
Vice President of Technical Services

Janice Jones
Vice President of Laboratory Operations

References

1. Aerotech Standard Operating procedure for Microbial Test Chamber Operation and Maintenance, 35-003.01
2. Aerotech Standard Operating Procedure for the Determination of Mycotoxins using HPLC With DAD and FLD Detectors, 05-012.00.
3. Aerotech Standard Operating Procedure for the Preparation of Swab, Bulk, CarpetChek, and Water Samples for Viable Fungal Analysis, 20-0096.00

VII. Table

Table I

Results Showing Effect of Ozone Treatment on *Aspergillus versicolor* and Associated Mycotoxins

Parameter	Pre Ozone	Post Ozone	Units
Viable Fungi	**3,400**	**<18**	CFU/cm^2
Aflatoxin B1	< 0.0010	*	µg/cm^2
Aflatoxin G1	< 0.0010	*	µg/cm^2
Citrinin	< 0.0050	*	µg/cm^2
Gliotoxin	< 0.025	*	µg/cm^2
Griseofulvin	< 0.00050	*	µg/cm^2
iso-Satratoxin G	< 0.0010	*	µg/cm^2
Ochratoxin A	< 0.0025	*	µg/cm^2
Ochratoxin B	< 0.0025	*	µg/cm^2
Patulin	< 0.0020	*	µg/cm^2
Penitrem A	< 0.0050	*	µg/cm^2
Roridin A	< 0.0020	*	µg/cm^2
Roridin E	< 0.0020	*	µg/cm^2
Roridin L-2	< 0.0010	*	µg/cm^2
Satratoxin G	< 0.0020	*	µg/cm^2
Satratoxin H	< 0.0020	*	µg/cm^2
Sterigmatocystin	< 0.00050	*	µg/cm^2
Verrucarin A	< 0.00050	*	µg/cm^2
Verrucarin J	< 0.0010	*	µg/cm^2
Zearalenone	< 0.0010	*	µg/cm^2

*Not Determined

VIII. Figures

Cultivation of Stock Organisms

Figure 1. *Aspergillus versicolor*

Drywall Preparation

Figure 2. Application of harvest cultures onto drywall

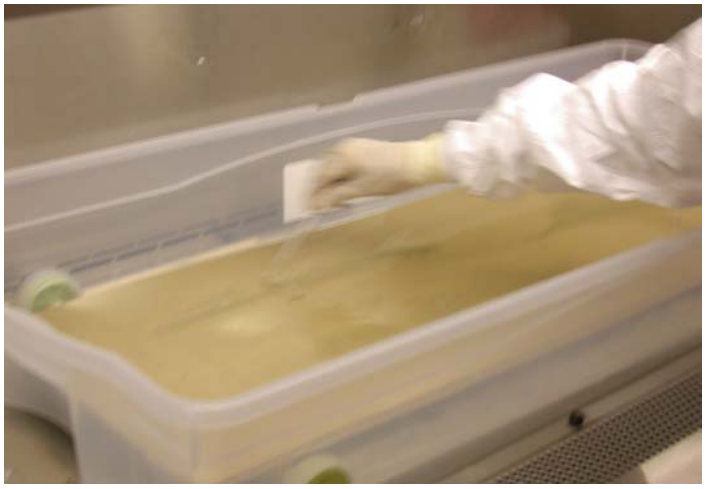


Figure 3. Streaking of cultures to ensure even distribution

Sampling

Figure 4. Sampling of viable *Aspergillus versicolor*

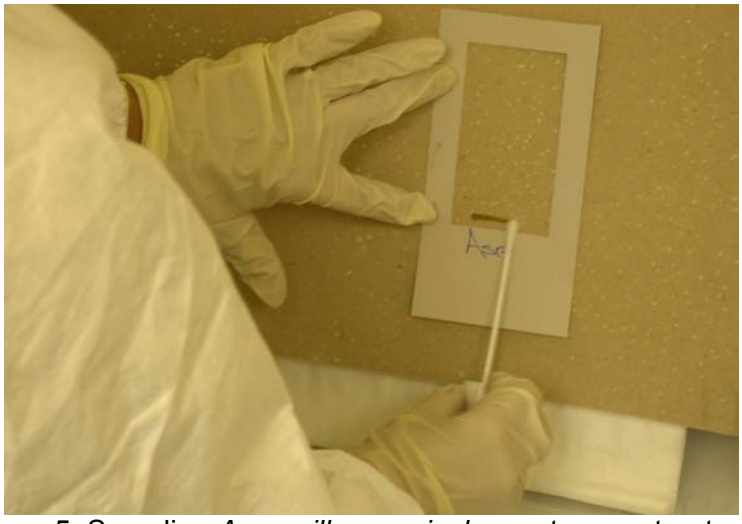


Figure 5. Sampling *Aspergillus versicolor* post ozone treatment

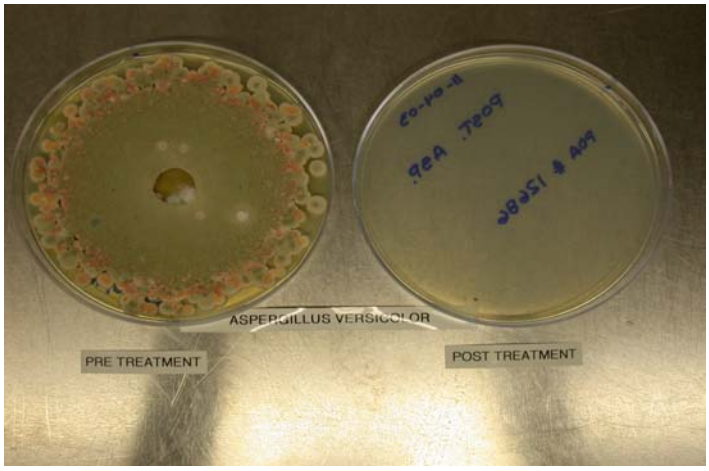
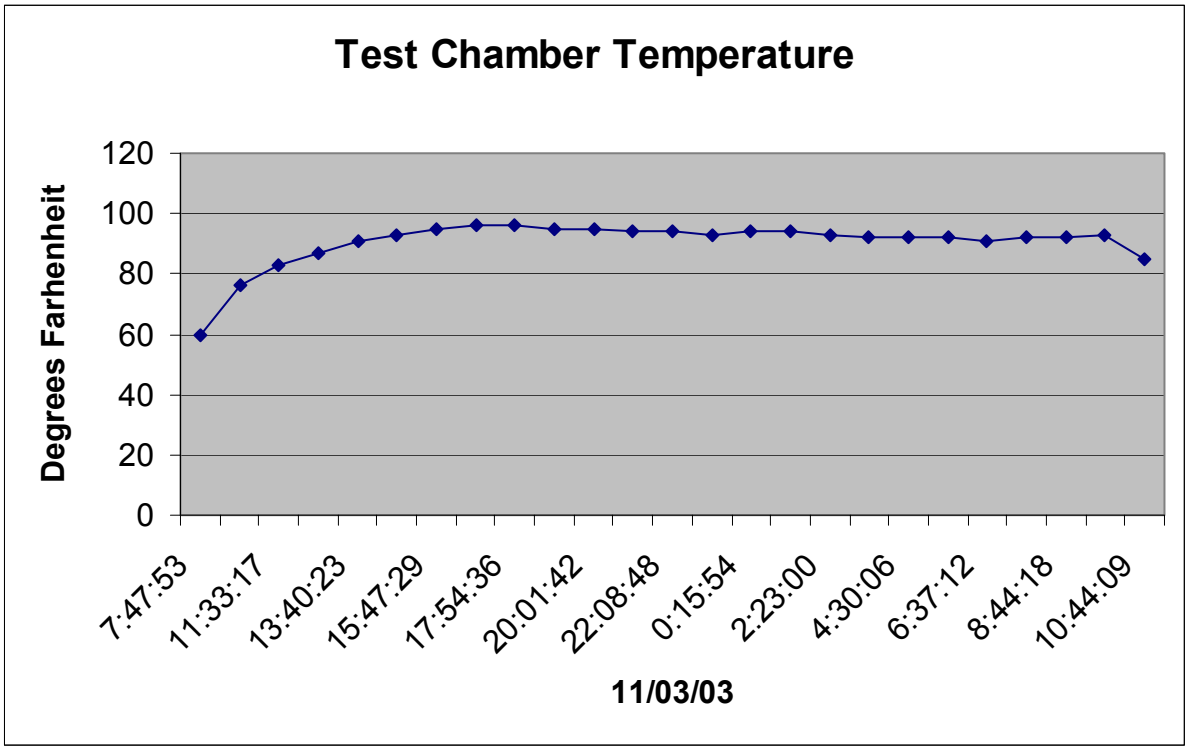
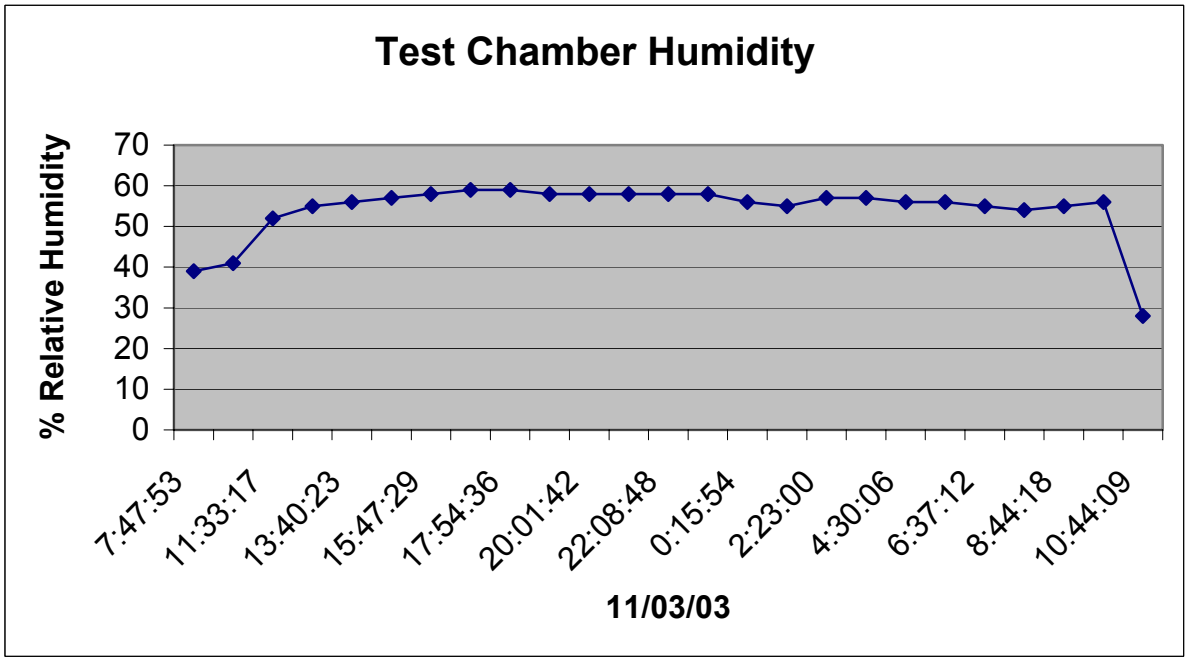


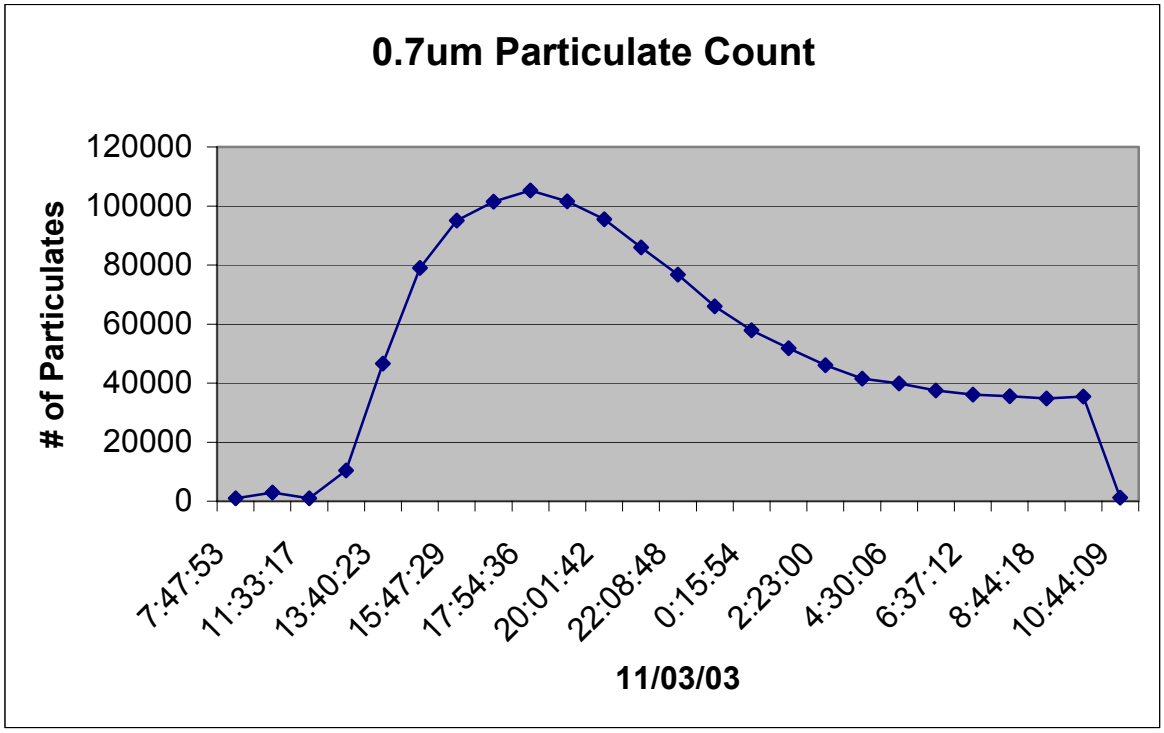
Figure 6. *Aspergillus versicolor* culture plate, Pre and Post ozone

IX. Appendices

Appendix A: Test Chamber Conditions



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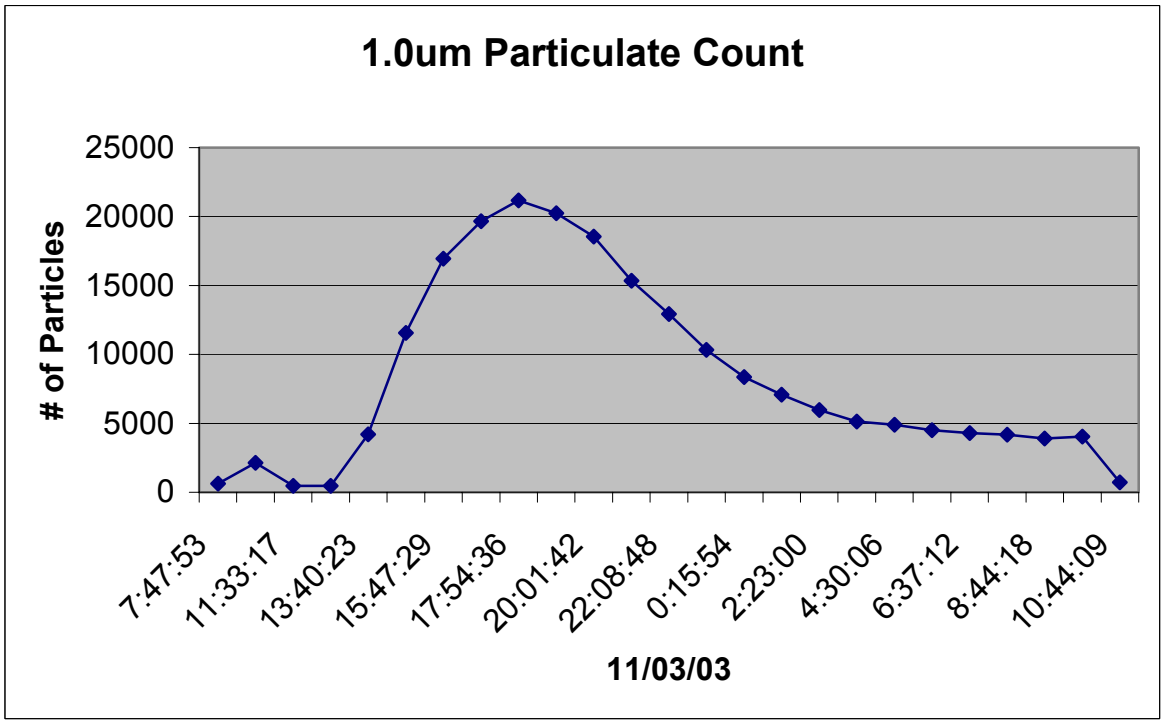
11/03/03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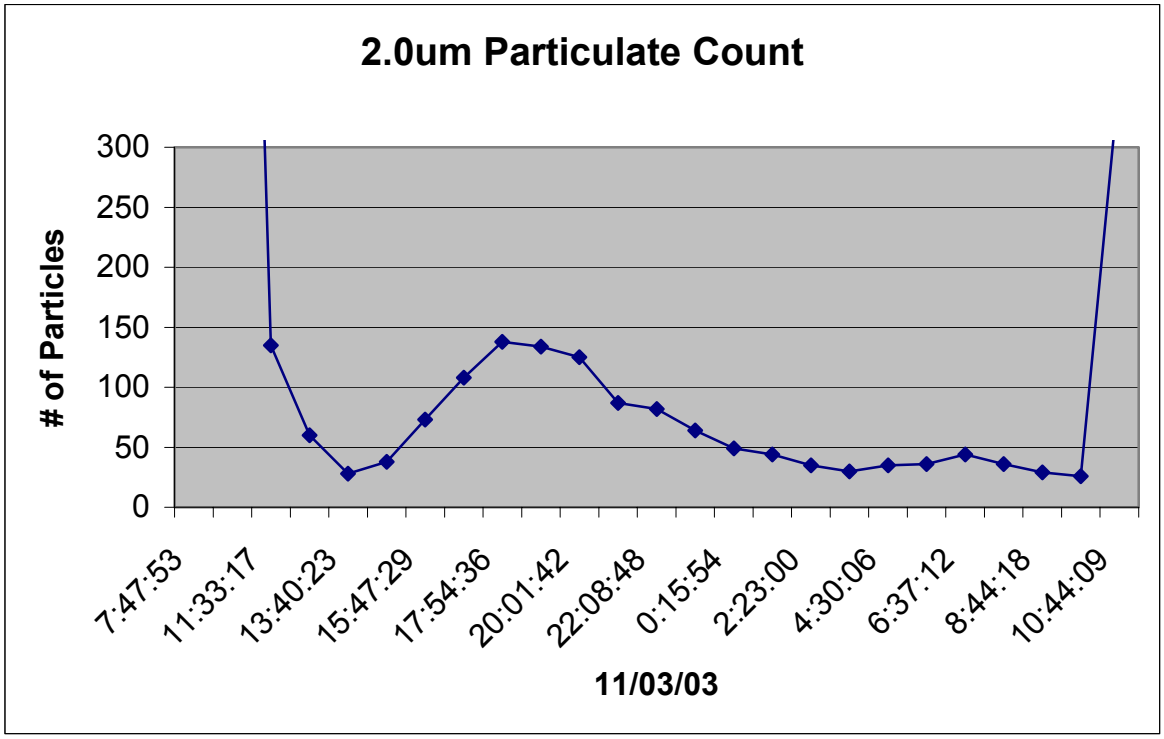
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