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Research Project for Zimek Technologies, LLC

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Background Information:
Mr. David Sparks, Executive Vice President of Research and Development for Zimek Technologies Management Company, contacted Microbac Laboratories, with an interest in determining, scientifically, the effectiveness of the micro environmental sanitation machine produced by Zimek Technologies, LLC ("Zimek") which produces negatively charged micro particles in delivering the microbicidal Zimek QD disinfectant wherever air flows. Zimek Technologies Management Company is the Manager of Zimek Technologies, LLC. The initial study was done in the Microbac Laboratory February 21, 2006 and was reported March 1, 2006. This second research project is a study of the effectiveness of the micro particle delivery system to mold samples on the bottom, sides and top of the test chamber.

Mr. Sparks manufactured a Plexiglas chamber 48 inches wide, 40 inches tall by 24 inches deep. Removable magnetic doors on the upper surface permit cleaning of the internal surfaces and permit placement of test specimens such as mold, bacteria and insects to the top, sides and bottom surfaces. Before use, the test chamber was cleaned with 70% isopropyl alcohol which was then allowed to evaporate. Two gallons of microbicidal Zimek QD were added to the patent pending micro particle producing equipment.

The heart of the supply system utilizes high voltage electrical oscillation at ultrasonic frequencies. A ceramic disc converts the electrical oscillation into mechanical oscillations which produce the negatively charged micro particle dry fog. [1] Ultrasonic nebulizers, like that utilized in the Zimek supply system, produce an average particle size of 1.7 microns with a low size of 0.9 microns and a high size of 3.0 microns and larger

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due to coagulation.[2] Bacteria are generally about 1 micron in length, mold spores are generally 2 to 5 microns in diameter and pollen generally ranges from 10 to 50 microns across.[3]

The smaller the particle size, the longer the mist remains in the air thereby allowing the microbicidal Zimek QD to destroy mold spores and bacteria in the air, on the ceiling and on the walls. As a reference point, 5 micron particles take 66 minutes to fall ten feet.[4]

The negatively charged micro particles move readily through a six foot long, two inch diameter hose to the inlet port in the lower back of the test chamber. The micro particles travel readily around many baffles set at 45 degree angles and enter the top of the test chamber. Standard sprays of 240 microns fall ten feet in six seconds and would be unable to pass through this rising vertical baffle system.[4] The micro particles travel easily to every cubic centimeter of the test chamber.

A two inch diameter hose at the top of the test chamber is draped inside an exhaust hood to prevent the escaping microbicidal micro particles from entering the laboratory.

Optical density within the test chamber is measured by placing a small flashlight at the top of a vertical 1 inch diameter PVC pipe shining up to an Extech Model EA30 light meter.

Preparation of Fungal Specimens:
Concentrated spores in vials were obtained from the Microbac Laboratory – Knoxville Division:
Aspergillus niger ATCC 16888 – 5.0×10^8 spores per mL.
Penicillium citrinum FRR 1841 – 1.34×10^9 spores per mL.
Stachybotrys chartarum VAMH 6417 – 2.15×10^8 spores per mL.

A one mL aliquot of each spore concentrate was diluted into a vial containing 99 mL of sterilized deionized water. A 0.5 mL aliquot was transferred to each of four petri dishes, with each petri dish containing Malt Extract Agar. A sterile, plastic spreader "hockey stick" was used to spread the spores evenly across each petri dish. The petri dishes were incubated for five days in the environmental chamber at 30° C and 97% relative humidity. Using sterile techniques, the spores were collected with sterile deionized water into a sterile125 mL flask. Glass beads inside the flask were used to break up the clumped spores on the vortexing machine. The spores were washed with sterile deionized water and centrifuged three times. Following the last centrifugation (10 minutes at 2,000 rpm) and removal of wash water, 10 mL of sterile deionized water was pipetted into each centrifuge tube.

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An aliquot of 0.3 mL was removed from the *Aspergillus* spore suspension. 0.1 mL was placed in each of three petri dishes containing Malt Extract Agar and spread across the entire surface.

An aliquot of 0.3 mL was removed from the *Penicillium* spore suspension. 0.1 mL was placed in each of three petri dishes containing Malt Extract Agar and spread across the entire surface.

An aliquot of 0.4 mL was removed from the *Stachybotrys* spore suspension. O.1 mL was placed in each of four petri dishes containing Malt Extract Agar and spread across the entire surface.

The ten petri dishes were incubated in the environmental chamber for 5 days at 30° C and 97% relative humidity.

Preparation of the Bacterial Specimen:
The bacteria culture employed in this test was obtained from the Microbac Laboratory – Venice Division stock cultures.
Staphylococcus aureus ATCC # 6538

A 0.1 mL aliquot of *Staphylococcus* stock culture was pipetted into each of two petri dishes containing PCA and spread evenly across the agar surface in each petri dish. Each dish was incubated 48 hours at 35° C.

Test Run:
An uncovered petri dish of *Aspergillus* culture was placed on the bottom of the test chamber in position "A" and the second was Velcro attached to the side position "D". The third *Aspergillus* culture served as the untreated control.

An uncovered petri dish of *Penicillium* culture was placed on the bottom of the test chamber in position "B" and the second was Velcro attached to the side position "E". The third *Penicillium* culture served as the untreated control.

An uncovered petri dish of *Stachybotrys* culture was placed on the bottom of the test chamber in position "C" and the second was Velcro attached to the side position "F". The third was Velcro attached, upside facing down, to the top of the test chamber in position "H". The fourth *Stachybotrys* culture served as the untreated control.

An uncovered petri dish of bacterial culture, *Staphylococcus aureus,* was Velcro attached to the side of the test chamber in position "G". The second *Staphylococcus* culture served as the untreated control.

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The two access doors on the test chamber top surface were both closed. The micro particle generator was turned on and within 7 seconds the "fog" entered the chamber from the top of the baffle. The fog had entered the main portion of the test chamber unhampered by the length of hose or the baffle chamber. The initial optical density was 31.50 and within three minutes was down to 0.40. After the 18 minute treatment time, the optical density was 0.12. Following the 30 minute dwell time, the optical density had increased to 26.2 as the micro particles had settled to the bottom of the test chamber. The exhaust hood was then turned on to remove remaining suspended micro particles from the test chamber.

The petri dish cultures were removed, covered and refrigerated. Three hours later the viable and non-viable fungal spores were collected, diluted and plated on Malt Extract Agar to determine spore counts. The *Staphylococcus* bacterial sample was collected, diluted and pour plated with bacterial Plate Count Agar.

Test Results:

Standard approved procedures established by AOAC and FDA/BAM were used in the collection of mold spores and bacteria and in their dilution into petri dishes with appropriate media and cultivation.[5, 6] The mold spores germinated and grew into colonies in an environmental chamber at 30° C and 97% relative humidity. The bacteria grew into colonies in the 35° C incubator.

The *Stachybotrys* control had a count of 50,000,000 viable spores per petri dish. The inverted top treated petri dish culture had a viable spore count of 1,400,000 (a 97.2% reduction). The side treated petri dish culture had a viable spore count of 1,000,000 (a 98.0% reduction). The bottom treated petri dish culture had a viable spore count of 500,000 (a 99.0% reduction).

The *Penicillium* control had a count of 640,000,000 viable spores per petri dish. The side treated petri dish culture had a viable spore count of 800,000 (a 99.8% reduction). The bottom petri dish culture had a viable spore count of 28,000 (a 99.99% reduction).

The *Aspergillus* control had a count of 22,000,000 viable spores per petri dish. The side treated petri dish culture had a viable spore count of 820,000 (a 96.27% reduction). The bottom petri dish culture had a viable spore count of 82,000 (a 99.63% reduction).

The *Staphylococcus* bacteria control had a count of 18,000,000,000 Colony Forming Units (CFU) per petri dish. The side treated petri culture had a count of 2000 Colony Forming Units (a 99.9999% reduction).

Conclusion:

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The Zimek micro particle delivery system of Zimek QD is effective in killing 96% to 99.99% of mold spores and bacteria growing in petri dishes attached to the top, sides and bottom of the Zimek test chamber over the 18 minute exposure period with 30 minute dwell time.

Reference Material:

1. MICO Importers produce the oscillating ceramic disc and are the proprietary suppliers to Zimek.
2. Sonaer Ultrasonics, 145 Rome Street, Farmingdale NY 11735.
3. McCrone Research Institute, Identification of Mold and Pollen.
4. Selecting the Correct Nozzle to Reduce Spray Drift, Iowa State University, IPM, and July 2001.
5. American Association of Analytical Chemists (AOAC) Official Method 966.23.
6. Federal Department of Agriculture/Bacteriological Analytical Manual (FDA/BAM) Chapter 3.

Respectfully submitted by:

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