



®

Microbac Laboratories, Inc.**Venice Division**

115 Corporation Way, Unit F

Venice, FL 34285

(941) 484-6508

Research Project for Zimek Technologies, LLC

Main Office: Roy Beckett, Chairman and CEO, Zimek Technologies Management Company, 13537 Granville Avenue, Clermont, FL 34711.

Branch Office: David Sparks, Executive Vice President of Research and Development, Zimek Technologies Management Company, 12713 Thonotosassa Road, Thonotosassa, FL 33592.

General Counsel: Kurt Grosman, General Counsel and CFO, Zimek Technologies Management Company, c/o The Grosman Practice, 5043 Winwood Way, Orlando FL 32819

Initiation of Project: February 21, 2006

Report Date: March 1, 2006

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 is reported March 1, 2006.

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 Zimek 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 due to coagulation.² Bacteria are generally about 1 micron in length, mold spores are 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.⁵ 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.

Pencillium 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 sterile 125 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 2000 rpm) and removal of wash water, 10 mL of sterile deionized water was pipetted into each centrifuge tube.

0.1 mL of each fungal spore suspension was pipetted into each of four Petri dishes. The drops were dried onto the bottom each petri dish upon 30 minutes in the 35° C incubator. One uncovered petri dish of each dried fungal spore suspension was placed on the bottom of the test chamber for each of the three tests times. The fourth petri dish of each dried fungal spore suspension was used as the control to determine the untreated fungal spore concentration.

Preparation of Bacterial Specimens:

The bacteria cultures employed in Test #1 (18 minute exposure time) were obtained from the Microbac Laboratory – Venice Division stock cultures.

Listeria innocua ATCC #33090
Salmonella enteritidis ATCC #13076
Escherichia coli ATCC #25922
Staphylococcus aureus ATCC #6538

A 0.1 mL aliquot of *Listeria* stock culture was pipetted into each of two petri dishes containing PCA (Plate Count Agar.) A sterile plastic spreader was used to spread the bacteria evenly across the agar surface in each petri dish. Each petri dish was incubated 48 hours at 30° C. One culture was used in Test #1 and the other was used to obtain the bacterial count per petri dish before treatment.

A 0.1 mL aliquot of *Salmonella* stock culture was pipetted into each of two petri dishes containing PCA and spread evenly across the agar surface in each petri dish. Each petri dish was incubated 48 hours at 35° C. One culture was held as a control while the other was treated in Test #1.

A 0.1 mL aliquot of *Escherichia* stock culture was pipetted into each of two petri dishes containing PCA and spread evenly across the agar surface in each petri dish. Each petri dish was incubated 48 hours at 35° C. One culture was held as a control while the other was treated in Test #1.

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 petri dish was incubated 48 hours at 35° C. One culture was held as a control while the other was treated in Test #1.

Test Run #1: (18 minute treatment)

An uncovered petri dish of *Aspergillus* spores was placed on the chamber bottom on position “A” along with an uncovered petri dish of *Listeria* culture. An uncovered petri dish of *Penicillium* was placed on the chamber bottom on position “B” along with an uncovered petri dish of *Salmonella* culture. An uncovered petri dish of *Stachybotrys* spores was placed on the chamber bottom on position “C” along with an uncovered petri dish of *Escherichia* culture and an uncovered petri dish of *Staphylococcus* culture. 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 32.25 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 24.2 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.

Results of Test Run #1:

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.^{6,7} 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 *Aspergillus* control had a spore count of 2,000,000 per petri dish. The treatment reduced this level to <2 spores per petri dish.

The *Penicillium* control had a spore count of 20,000,000 per petri dish. The treatment reduced this level to <2 spores per petri dish.

The *Stachybotrys* control had a spore count of 640,000 per petri dish. The treatment reduced this level to <2 spores per petri dish.

The *Salmonella* control had a spore count of 57,000,000,000 CFU (colony forming units) per petri dish. The treatment reduced this level to <2 CFU per petri dish.

The *Listeria* control had a count of 12,500,000,000 CFU per petri dish. The treatment reduced this level to <2 CFU per petri dish.

The *Escherichia* control had a count of 12,000,000,000 CFU per petri dish. The treatment reduced this level to 110 CFU per petri dish.

The *Staphylococcus* control had a count of 12,000,000,000 CFU per petri dish. The treatment reduced this level to 110 CFU per petri dish.

Test #2: (16 minutes treatment)

An uncovered petri dish of *Aspergillus* spores was placed on the chamber bottom on position “A”. An uncovered petri dish of *Penicillium* spores was placed on the chamber bottom on position “B”. An uncovered petri dish of *Stachybotrys* spores was placed on the chamber bottom on position “C”. 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 28.84 and within three minutes was down to 0.40. After the 16 minute treatment time, the optical density was 0.12. Following the 22 minute

dwelt time, the optical density had increased to 12.45. The exhaust hood was then turned on to remove remaining suspended micro particles from the test chamber.

Results of Test Run #2:

The *Aspergillus* control had a spore count of 2,000,000 per petri dish. The treatment reduced this level to <2 spores per petri dish.

The *Penicillium* control had a spore count of 20,000,000 per petri dish. The treatment reduced this level to <2 spores per petri dish.

The *Stachybotrys* control had a spore count of 640,000 per petri dish. The treatment reduced this level to <2 spores per petri dish.

Test Run #3: (13 minute treatment)

An uncovered petri dish of *Aspergillus* spores was placed on the chamber bottom on position “A”. An uncovered petri dish of *Penicillium* was placed on the bottom of the test chamber on position “B”. An uncovered petri dish of *Stachybotrys* was placed on the bottom of the test chamber on position “C”. 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 unhampered by the length of hose or the baffle chamber. The initial optical density was 27.00 and within three minutes was down to 0.40. After the 13 minute treatment time, the optical density was 0.11. Following the 22 minute dwell time, the optical density had increased to 23.92. The exhaust hood was then turned on to remove remaining suspended micro particles from the test chamber.

Results of Test Run #3:

The *Aspergillus* control had a spore count of 2,000,000 per petri dish. The treatment reduced this level to <2 spores per petri dish.

The *Penicillium* control had a spore count of 20,000,000 per petri dish. The treatment reduced this level to <2 spores per petri dish.

The *Stachybotrys* control had a spore count of 640,000 per petri dish. The treatment reduced this level to <2 spores per petri dish.

Conclusion:

The negatively charged micro particles deliver the Zimek QD microbicide very efficiently to the bottom surface of the test chamber when allowed to settle after 18, 16 and 13 minute generation times.

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, July 2001.
5. Selecting the Correct Nozzle to Reduce Spray Drift, Iowa State University, IPM, July 2001.
6. American Association of Analytical Chemists (AOAC) Official Method 966.23.
7. Federal Department of Agriculture/Bacteriological Analytical Manual (FDA/BAM) Chapter 3.

Respectfully submitted by:



Kayse M. Hasiak
Laboratory Director
Microbac Laboratories, Inc. – Venice Division
115 Corporation Way, Unit F
Venice, Florida 34285