DESIGN AND EVALUATION OF A PORTABLE ULTRA-VIOLET MICROBIAL DISINFECTION CHAMBER

by

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(ABSTRACT)

With the increasing interest in the indoor air environment over the last 30 years has come the need for control devices that can improve the quality of air that people breath. To answer this need, many devices have been developed and are currently in use. This thesis utilizes Ultra-violet light to eradicate the bacteria. A portable unit was constructed that contains the light bulb and fan. Two different trials were conducted: Controlled Laboratory trials and Fish Culture Room Trials. The Controlled Laboratory Experiments were conducted in order to test the effect that the Ultra-violet unit had on known cultures of bacteria while the Fish Culture Room Trials were conducted to test the chambers affect on a real indoor situation. The Ultra-violet unit was effective in eradicating the known cultures that were tested, and was less effective in the real indoor setting. As the density of bacteria increased, the effectiveness of the chamber increased, for the real indoor setting. The contact time of the chamber could be adjusted to improve effectiveness. More testing is necessary to fully evaluate the potential of a portable Ultra-violet disinfection chamber.

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CHAPTER I. INTRODUCTION

The concern over the quality of the air that we breathe has increased over the last twenty years. With Americans spending an average of 90 percent of their time indoors and the continuing energy consciousness of building construction, indoor air pollution problems have succeeded in separating themselves from the all encompassing topic of "Air Pollution"(1). With this separation came increased scrutiny and effort on behalf of regulators, building owners and the occupants themselves to better understand and deal with indoor air problems. In addition to an increase in research delving into the causes of indoor air problems came an increase in the development of control devices.

The use of UV radiation as a control device has been studied since the 1940's and has proven to be effective. New understanding of the factors that cause indoor air problems and advancement in the design of ultraviolet (UV) bulbs have resulted in a reevaluation of the role that UV radiation can play in the prevention of some indoor air problems.

A myriad of pollutants have been found to cause health problems for building occupants. Stolwijk (2) found that microbial contamination causes five percent of indoor air problems. The Occupation Safety and Health Association (OSHA) estimates that "30 percent of indoor workers are affected by poor indoor air quality" (3); this has led OSHA to develop a Proposed Indoor Air Pollution Rule. This rule would require building owners to develop an Inspection and Maintenance program for all commercial buildings. Preventive measures such as an Inspection and Maintenance program are a good step, however, further controls are needed to insure that building occupants have a healthy environment to work in. Control

methods that have been tried in the past include High Efficiency Particle Adsorption (HEPA) filters, increasing ventilation rates and UV radiation.

Past research that explored the use of UV radiation typically attempted passive disinfection of air in corridors and rooms. Although those studies showed that UV radiation was successful in killing bacteria in the air, they were conducted over forty years ago using bulbs that were not as effective or powerful as those manufactured today. The risk of exposure to the UV radiation and the inability to insure the disinfection of all the air in a room were other disadvantages. A new concept that would address these disadvantages while utilizing the most current UV bulbs could be an answer to some of the indoor air pollution problems associated with microbiological contamination.

This thesis was designed to explore such a concept with the following objectives:

- Design and build a portable UV unit that can effectively reduce the amount of bacteria found in indoor air.
- Determine the UV unit's ability to effectively eliminate known types of bacteria in an air medium.
- Determine the UV unit's ability to reduce the amount of bacteria found in a typical building environment.

CHAPTER II. LITERATURE REVIEW

In the past few years there has been a renewed interest in the prospects of using UV radiation to control indoor air pollutants. Most of the research dealing with this renewed interest is currently underway or in the process of publication. The concept that this thesis has been based on - a portable UV disinfection unit with mechanical air transport - has yet to be explored. A review of the literature that has been utilized in the planning and execution of this thesis can be grouped into the following categories:

- Indoor Air Pollution Problems and Impending Regulations
- Ultraviolet radiation: description and health concerns
- Past and Present UV radiation research
- Aerosol Sampling: Sampler types, description, operation
- Microbiology

The following section, deals with each of these categories.

Indoor Air Pollution Problems and Impending Regulations

Indoor Air Pollution (IAP) problems have been on the rise over the last two decades. The energy crisis in the 1970's started a steady rise in the cost of energy. This rise has initiated a movement towards energy conservation in building construction and operation. Reducing the amount of infiltration air entering buildings was one major step taken and with

the increasing reliance on mechanical ventilation building operators began reducing the amount of makeup air that is brought into the Heating Ventilation and Air Conditioning system (HVAC). These three steps have reduced the cost of heating and air conditioning a building; however, they have also increased the chance for indoor air problems to develop (4,5). Reducing the amount of infiltration air and makeup air for the HVAC has reduced the dilution and removal of pollutants. Hines *et al.* (6) gathered and tabulated the results of five groups of researchers that had compared the indoor concentrations of the most common indoor pollutants to the outside concentration, with surprising results. Thirty-eight of the forty-six pollutants studied had higher indoor concentrations; the differences ranged from less than 1% to over 800 times higher with the majority of the indoor levels about two times higher. Some of the health effects associated with indoor air pollutants include infections such as Legionnaire's disease and acute respiratory disease, hypersensitivity or allergic disease, headaches, asthma, and central nervous system symptoms (7). In addition to the cost of treating health problems are the costs incurred due to loss in productivity and morale.

Indoor air pollution has become so much of a concern that the Occupation Safety and Health Administration (OSHA) is currently in the process of developing an Indoor Air Quality Rule and the Environmental Protection Agency (EPA) also is considering an indoor air quality regulation. By their own research, OSHA estimates that 21 million employees are potentially effected by poor indoor air quality (3). Although neither agency has implemented a plan, after reading OSHA's proposed rule an attorney and legal editor for the Environmental Practice Group has stated that "The IAQ proposal is sweeping in scope and effect." (8). Regardless of the ultimate regulations building owners and employers have realized that they must take

an active role in the maintenance of their work environments. This realization has led to the search for the most cost effective way of controlling and correcting indoor air problem. Using UV radiation to control these problems is one such answer.

Ultraviolet radiation: description and health concerns

Ultraviolet wavelengths are shorter than those of visible light and are therefore invisible to the human eye. The wavelength of UV light falls between 10 and 450 nm with the most effective wavelength for killing microorganisms is at 253.7 nm. The wavelengths in this area are referred to as UV-C, they are the shortest of the UV wavelengths. The nucleic acids found in the DNA of exposed bacteria are the key to understanding the disinfecting properties of UV radiation. Nucleic acids absorb the UV radiation strongly between the wavelengths of 250 and 260 nm. This causes bonds to form between adjacent thymines in the DNA chains; these thymine dimers inhibit correct replication of the DNA during reproduction of the cell (9). Other dimers have been implicated in addition to the above mentioned thymine dimer, such as the pyrimidine-pyrimidine cyclobutane dimer (10). Sunlight contains all four types of UV wavelengths - UV-A, B, C, and D; however, the shorter, UV-C wavelengths (the most effective in killing microorganisms) are absorbed by the upper atmosphere.

The health effects caused by exposure to UV radiation apply to the eyes and skin. Short-term exposure causes mild irritation to the eyes and redness of the skin. Long-term exposure can lead to cataracts and burns and may lead to skin cancer. An ordinary piece of

glass will block the harmful rays to allow observation of the UV bulb. In order to be effective at killing microorganisms, UV bulbs have been designed to emit wavelengths between 250-260 nm.

Although UV radiation at the 253.7 nm wavelength is very effective in killing microorganisms, there are some limitations. The UV radiation is not very penetrating; the wavelengths can be blocked, due to absorption by such items as paper, regular glass, or dust. The microorganisms must come in direct contact with the radiation to be affected. Due to these limitations, placement of the UV bulb is very important in addition to the size of the space that the bulb must treat. Phillips® recommends that the bulb be positioned perpendicular to the flow of air and that a reflective coating be applied to the surrounding walls to increase reflection.

Past and Present UV radiation research

The background information regarding UV bulbs was obtained from conversations with Phillips Lighting Company Technical Support Line and literature supplied by that support staff (11) . This information was gathered from the work of Harvey Rentschler. Dr. Rentchler conducted his research in the 1940's for Westinghouse. During this same period other researchers were exploring the use of UV radiation and its effect on microorganisms (12,13). The main impetus of this research was to analyze the effect that UV radiation had on microorganisms and the effectiveness of using UV lights to kill microorganisms in a passive approach. The lights were installed in hallways, operating rooms, and in medical waiting rooms. This approach had some drawbacks that hindered the acceptance of UV

disinfection. Exposure to the harmful radiation was a major concern, the efficiency depended on the amount of air that reached the lights, and the bulbs used at that time were not very powerful. Passive use of UV lights could be found in hospitals and barber shops and UV disinfection enjoyed limited success in water disinfection. Widespread acceptance and use of UV radiation for air disinfection, however, did not ensue. Sterile-Aire USA in Cerritos, California, an indoor air quality firm, attributes this to the UV bulbs output and stability (14). New technology has improved the bulbs so that they are effective in cold and hot environments and produce up to five times as much radiant output. When these bulbs are used in conjunction with modern filters such as the HEPA filter, the interference from particulates, which decreases the efficiency of the bulbs, is reduced.

The advances in the manufacturing of UV bulbs has sparked a renewed interest in their use. Two companies that are currently marketing this technology are Sterile-Aire USA and Air Handlers Clean Air Concepts Inc. of West Palm Beach, Florida. Both companies have incorporated their devices to work with the HVAC system in residential or commercial settings and both claim that their products will effectively kill microorganisms. Independent research exploring the effectiveness of both products needs to be performed to test their ability to function effectively under normal operating conditions. This would include the effect that dust or humidity has on their efficiency along with different temperature changes and microorganism types and concentrations.

A number of research projects have been undertaken in the last few years. In 1994 the National Tuberculosis Coalition initiated a study to explore the possibility of using UV lamps to prevent the spread of tuberculosis, their findings are expected late in 1999 (15).

LightStream of Alameda, California in conjunction with the Electric Power Research Institute (EPRI) is researching the possible use of UV light with photocatalytic reactors to destroy VOC's, odors, carbon monoxide, nitrogen oxides, ozone and microorganisms; their first product is expected in 1996 (16).

Aerosol Sampling: Sampler types, description, operation

Along with the quest for the understanding of microorganisms in air has been the search for the perfect sampler to collect those organisms. The samplers that have been developed include impingers, filter cassettes, slit cascade impactors, high-volume filtration, high-volume electrostatic, sieve impactors, and centrifugal impactors. Each type of sampler has its benefits and limitations. Depending on the type of sample being collected, the data desired and the location of sampling, one sampler would be preferred over another. One of the most popular samplers has been the Andersen Cascade Impactor (17). The Anderson sampler is widely accepted as the standard sampler for collecting viable aerosols (18).

The following is the description of the Six-Stage Viable Particle Sampler from the Operating Manual for Andersen Samplers Inc., Atlanta, Georgia.

> "The Andersen 1 ACFM Viable Particle Sampler is constructed with six aluminum stages that are held together by three spring clamps and sealed with O-ring gaskets. Each impactor stage contains multiple precision drilled orifices. When air is drawn through the sampler, multiple jets of air in each stage direct any airborne particles toward the surface of the agar collection surface for that stage. The size of

the jet orifices is constant within each stage, but are smaller in each succeeding stage. The range of particle sizes collected on each stage depends on the jet velocity of the stage and the cutoff of the previous stage. Any particle not collected on the first stage follows the air stream around the edge of the Petri dish to the next stage. Each stage contains 400 orifices with diameters ranging from 1.81 mm on the first stage to 0.25 mm on the sixth stage."

The sampler is then attached to a pump that is calibrated to draw 1 Actual Cubic Foot per Minute (ACFM). It is critical, and recommended, that the pump be calibrated prior to each use.

Despite the Andersen Sampler's widespread use and acceptance, it has limitations (19). The even distribution of particles across each stage is important if the sampler is expected to collect a representative sample. Although stages 3 - 6 distribute particles evenly, stages 1 and 2 vary in the distribution. Furthermore, the sampler is designed, and is quite efficient at collecting particulates between the sizes of 0.5 μm and 5 μm. Particles outside of this range tend to be collected on the different stages. For example, particles 12 μm and larger are often collected on the first stage's agar plate.

CHAPTER III. METHODS AND MATERIALS

Ultraviolet Chamber Design

A portable UV chamber that utilizes mechanical air transport while eliminating the risk of exposure to harmful radiation and effectively eliminate bacteria was the overall goal of the design phase of the research.

The components of the Ultraviolet chamber (see Figure 1.) were as follows:

Fan	-	Radio Shack Four inch Cooling Fan 65 CFM 120 VAC No. E 89061
Ultraviolet Light	-	Philips Germicidal Sterilamp 15 Watt 16 7/8 inch Single Pin Model G10T51/2L
Ballast	-	Advance Transformer Co. Chicago, Ill 120 Volts 60 Hertz 425 mA 0.55 Amps Catalog Number SM-140-S-TP

The chamber consisted of a 24 inch cylinder of 14 gage steel with an 8 inch diameter, resulting in a volume of 0.698 ft^3. The chamber was lined with aluminum foil held in place with clear packing tape to eliminate short-circuiting. The purpose of the aluminum foil lining was to reflect the UV rays back toward the flow of air. Open-ended boxes 12 x 12 x 5 inches were placed on both ends of the chamber. These boxes fit over the ends of the cylinder allowing the air flow to exit the chamber while reducing the amount of UV rays that were emitted. The end boxes were designed so that the air flow would not be constricted, this was accomplished by making the area of the end boxes twice the size of

Figure 1 Ultra-violet Chamber - Page 11 (11.jpg 44 KB)

the chambers opening. This design, while reducing the amount of radiation that was emitted, did not eliminate it. Any commercial application would need to contain additional safeguards (The primary focus of this research was to evaluate the effectiveness of such a device, allowing the refinement of the design to be performed in later research). In the center of the box covering the exhaust end was a one inch hole that was drilled to provide access for the sampler.

A Phillips Germicidal Sterilamp Slimline UV bulb was mounted along the length of the cylinder. The space between the lamp and the cylinder wall was filled with closed cell foam and sealed with caulk to prevent short circuiting. Holes were drilled through the wall to facilitate the power cords for the bulb and fan.

A four-inch Radio Shack® Cooling Fan was mounted in the intake side of the cylinder. The square housing of the fan was fitted into a circular piece of Styrofoam® eight inches in diameter, which was then mounted in the intake side of the cylinder, held in place with and sealed with caulk.

Aerosol Samplers

The aerosol sampler used was the Andersen 1 cfm Viable Sampler (Andersen Inc., GA) on loan from the Aberdeen Proving Grounds at Fort Dietrich, Maryland. Typically, glass petri dishes supplied with the samplers are used in the collection of particles. Due to the sampling schedule for this thesis and the accepted use of plastic petri dishes (18,20-22), 100

x 15 mm plastic petri dishes, supplied by VWR Scientific Products, Bridgeport N.J., were used. The type and amount of media used will be discussed in a later section.

To ensure proper differentiation of particles by the sampler, a pump must be attached that pulls 1 ACFM of air. A Gast vacuum pump was used with the Andersen Sampler, and were calibrated on a daily basis using a Sprague bellows type dry test meter. In addition, the dry test meter was periodically calibrated using a GCA/Precision wet test meter. Calibration bench sheets for the dry test meter are contained in Appendix B. By adjusting the needle valves located on the pumps, they were calibrated to 1 ACFM ±1%. The two samplers were operated side-by-side prior commencement of the experiments. This was done to see if their was a significant difference in the amount of bacteria that each sampler collected. The two samplers were not found to have a significant difference in collection efficiency; the statistical data is contained in Appendix C. Each sampler was fitted with an 18 inch plastic tube with a one inch diameter that fit over the inlet cone. One sampler was placed at the inlet of the ultra-violet chamber and the other at the outlet with the plastic tube fitting through the hole in the end box.

Temperature and Relative Humidity Measurement

Temperature was measured using a Fluke Digital thermometer. This meter has the capacity to read two separate temperatures simultaneously, and was calibrated using a ERTCO brand NIST thermometer owned by Olver Laboratories Inc.. One lead from the

Fluke thermometer was attached to the inlet end and one to the outlet of the ultra-violet chamber.

Humidity measurements were conducted using a Bacharach Inc., Pittsburg, PA, sling psychrometer. The humidity was measured at a point adjacent to the inlet end of the UV chamber directly prior to each sampling run and was recorded.

Microbiological Methods

A wide variety of microorganisms have been implicated as causing or contributing to indoor air problems. Harriet A. Burge's book - *Bioaerosols* (23) is a good reference on this topic. The laboratory experiments that were conducted for this thesis were designed to test known types of bacteria that were normally found in indoor environments. *Eschericia coli* (*E. coli*) and *Pseudomonas* were the two bacteria that were chosen (24). By keeping the number of bacteria being tested at a minimum, it was possible to conduct a larger number of trials with each bacteria. Problems surrounding the aerosolizing of the *Pseudomonas* bacteria prevented it's use in testing (discussed in detail in Appendix A). At the time that the problem with *Pseudomonas* developed it was not possible to obtain another bacteria and test it due to the time constraint imposed by the loan of the Andersen Impactors. For this reason, a bacteria was chosen that has been commonly used in microbiology laboratories - *Enterobacter aerogenes* (*E. aerogenes*).

A myriad of bacteria and fungus can be expected in a location like the Fish Culture Room; where large amounts of fish are being bred and where the tanks are being aerated by

diffusers and filters, thus providing moisture, food, aerosolization and constant temperature for bacteria and fungus to thrive. For this reason, an agar that is well suited for bacteria collection and provides good general support for growth of microorganisms is recommended (18). Trypticase Soy Agar was chosen for the trials conducted in the Fish Culture Room (25). Since pure cultures of bacteria were being used in the Laboratory trials, agars that were specific for the type of bacteria being tested would reduce the chance of any extraneous bacteria from being included in the enumeration, if any other bacteria were present. LES-Endo agar was used for the *E. coli* trials due to it's specific support of coliform bacteria growth (26). Standard Methods Plate Count Agar was used for the *E. aerogenes* trials; due it's late substitution into the testing it was not possible to acquire an agar that was specific for *E. aerogenes*. Standard Methods Plate Count Agar is a general purpose agar capable of supporting the growth of *E. aerogenes*.

The microbiological methods will be discussed in the following two sections:

- Media Preparation
- Andersen Sampler

Media Preparation

All microbiological work was performed at Olver Laboratories in Blacksburg, Virginia. Olver Laboratories is certified by the State of Virginia to perform microbiological testing and is equipped with all of the necessary equipment needed to perform the testing and media preparation that was required by this thesis. Sections 9030B, Laboratory Apparatus Equipment Specifications and 9050A, Preparation of Culture Media, *Standard Methods for*

the Examination of Water and Wastewater (26) were two specific references that were used by the laboratory to equip and operate the microbiology laboratory.

Different agars were used depending on the sampling site or bacteria type. The two types of agar and the types of sampling that they were used for were:

Trypticase Soy Agar	-	Control Experiments Fish Culture Room at Olver Laboratories Inc. *E. aerogenes* Laboratory Experiments
LES Endo Agar	-	*E. coli* Laboratory Trials

* Both agars were supplied by BBL.

Both of the agars were prepared according to manufacture's specification. The Trypticase Soy Agar was then autoclaved at 121ºC for 15 minutes in 500 mL, screw-type Erlenmeyer flasks. The final pH of the agar was compared with the manufacturer's recommended ranges; 7.3 ± 0.2 for the Trypticase Soy Agar. The M-endo LES agar was boiled with frequent agitation, after boiling the pH of the agar was compared to the manufacturer's recommended range of 7.2 ± 0.2.

In order to preserve the correct distance between the inlet of each stage and the agar impaction surface, 45 mLs of agar was dispensed into each plate. This amount of agar was required in order to maintain the ability of the sampler to accurately fractionate the particles in the air stream.

A 100 mL graduated cylinder that had been calibrated using a Class A Pyrex graduated cylinder and then autoclaved, was used to measure the agar before being dispensed into the petri dishes. The agar was then dispensed into 100 x 15 mL plastic petri dishes. After allowing the plates to cool they were inverted and placed into a 35º C incubator to

check the sterility of the plates. This incubation period lasted for 24 hours, after which the plates were checked for any growth. The whole batch of agar was discarded if any plates showed bacterial growth. Usable plates were then wrapped in a plastic bag and stored in a <4º C refrigerator.

Following the sterility check each batch of agar was tested for the ability to grow bacteria. One plate from each batch was inoculated with *E. coli* grown from a certified pure culture supplied by DIFCO. Samples taken for this thesis utilized plates that were less than 72 hours old.

Andersen Sampler

The six-stage Andersen Cascade Sampler was prepared for sampling in the following manner. Prior to each trial, every stage of the sampler was inspected to ensure that the holes were not clogged. Each stage was then swabbed with 95% Isopropyl alcohol and reassembled with the other stages. An agar plate labeled with the date, trial number, and stage number was placed in the sampler. One sampler was labeled "Inlet" and was placed at the inlet end of the UV chamber for every run performed for this thesis, with the other one being labeled "Outlet" and was used in that position for every run. After the samples were taken the lids were reattached to the corresponding bottoms. The plates were then inverted and incubated for 48 hours at 35º C ± 0.5. At the end of the incubation period the number of colonies formed on each stage was counted and recorded. All counts were performed by the author.

Experiment Setup and Procedures

The UV chamber was tested under two conditions; a controlled laboratory setting and an uncontrolled location. The latter was chosen to test the chamber's ability to eradicate a mixed culture of bacteria occurring naturally in a location that closely resembles where the chamber would be operated commercially. The laboratory setting was chosen in order to validate the chamber's effectiveness using pure cultures of bacteria at specific concentrations, in a controlled environment. In addition to these two locations, a control experiment was conducted to measure the effect, if any, that the UV chamber had on bacteria without the application of the UV light.

The first section describes the Control experiments with the following topics being covered:

- Laboratory Test Apparatus
- Bacteria Preparation
- Experimental Procedures

Control Experiments

Test Apparatus

Due to the use of pure cultures of viable pathogenic bacteria a fume hood was used for the all the laboratory experiments. The UV chamber, Andersen Samplers, and aerosol generator were placed inside the confines of the fume hood.

A five-gallon plastic bucket with a lid was used as the aerosol generation chamber (see Figure 2.). Two, two-inch holes were drilled in the lid with eight-inch long PVC pipe being inserted into each hole. The two pieces of PVC pipe projected one inch above the lid. An 1/8 inch hole was drilled through the lid to allow an air line to be inserted into the chamber. One end of the air line was fitted with a one inch ceramic air stone. A Second Nature® Aquarium pump was connected to the other end of the air line to supply the air required to generate the aerosol. The air stone rested in a 500 mL glass container positioned at the bottom of the aerosol generation chamber. The aerosol exited the generation chamber via a four-inch opening in the lid that was connected to a ten-inch flexible plastic tube, which was attached to a cone with one four-inch opening and one eight-inch opening that was in turn attached to the inlet end of the UV chamber.

The inlet cones of the two Andersen Samplers were fitted with a two-inch plastic tube eighteen inches long. The plastic tube on one sampler was inserted into a two-inch hole in the ten-inch flexible plastic tubing that connected the aerosol generator to the UV chamber, drawing it's sample from the aerosol stream prior to entering the UV chamber. The plastic

Figure 2 Aerosol Chamber Page 20 (20.jpg 33.6 KB)

tube on the remaining sampler was inserted into the hole that was in the center of the open ended box attached to the outlet end of the UV chamber.

Bacteria Preparation

The pure cultures of bacteria that were used in the Control experiments were supplied by DIFCO, in disk form. Two types of bacteria were tested; *E. coli* and *E. aerogenes*. Initial solutions of the two bacteria were prepared in the following manner:

An *E. coli* bacterial disk was placed in a Milk Dilution Bottle containing 50 mL of triple strength Lauryl Sulfate Broth and 100mL of buffer water, made according to specifications found in Section 9050C, Media Specifications, *Standard Methods for the Examination of Water and Wastewater* (26). The Lauryl Sulfate Broth was prepared according to Section 9221, Multiple-tube Fermentation Technique for Members of the Coliform Group, *Standard Methods for the Examination of Water and Wastewater* (26). This solution was incubated at 35º C ± 0.5 for 48 hours and labeled "*E. coli* Stock Solution". *E. aerogenes* was prepared in the same manner as the *E. coli* and labeled "*E. aerogenes* Stock Solution". Both bacterial stock solutions were initiated 48 hours prior to use in the desired trials and were kept in the 35º C incubator until the trials were initiated.

Experimental Procedures

The following list of steps shows the typical procedure for the two bacteria that were tested.

1. The UV chamber was positioned inside the fume hood with the aerosol generator and set up according to the description mentioned in the Laboratory Apparatus section.
2. The two Andersen Samplers were sterilized using the procedure mentioned previously in the Microbiological Methods. The sterility of the two Andersen Samplers was then tested by placing two agar plates (one in stage two and on in stage five) in the reassembled Sampler for three minutes, with the pumps off.
3. All six stages of both Andersen Samplers were loaded with agar plates and the sampler was reassembled. The plastic tubes attached to the inlet cones of the samplers were then positioned in the manner described in the previously mentioned Laboratory Apparatus section.
4. The temperature of was taken using the digital Fluke thermometer. The Relative Humidity was then taken using the Bacharach sling psychrometer. Both of these measurements were recorded on the date sheet for that test.
5. The concentration of bacteria for the test was prepared and poured into the glass dish inside the aerosol generator. The aerating pump, fan and UV light were turned on for three minutes.

6. After the three minute period expired, the Andersen Samplers were simultaneously turned on for the desired amount of time.
7. After the Andersen Samplers were turned off the agar plates were removed and the lids to the petri dishes were reattached.
8. Steps 2 through 6 were then repeated until all trials for that test were performed.
9. Following the last replicate all of the agar plates were placed in the incubator at the same time. After 48 hours of incubation the colonies on the plates were counted and recorded.

A total of six trials using *E. coli* were conducted on two separate dates. The concentration used and the length of time that each replicate was conducted is presented in Table 1. Six experiments were conducted using *E. aerogenes* on two separate dates. The concentration used and the length of time that each replicate was conducted is presented in Table 2.

Table 1. *E. coli* Control Experiment Characteristics

DATE	TRIAL NUMBER	STOCK SOLUTION[1] (mL)	BUFFER WATER[2] (mL)	TRIAL DURATION
1/18/97	1	0.5	499.5	6 minutes
1/18/97	2	10	490	1.25 minutes
1/18/97	3	15	485	45 seconds
1/19/97	1	0.5	499.5	6 minutes
1/19/97	2	10	490	1.25 minutes
1/19/97	3	15	485	45 seconds

Note:

1. See page 21 of the Methods section for a description of the Stock Solution.
2. See page 21 of the Methods section for a description of the Buffer Water.

Table 2. *E. aerogenes* Control Experiment Characteristics

DATE	TRIAL NUMBER	STOCK SOLUTION[1] (mL)	BUFFER WATER[2] (mL)	TRIAL DURATION
1/25/97	1	0.5	499.5	6 minutes
1/25/97	2	10	490	1.25 minutes
1/25/97	3	15	485	45 seconds
1/26/97	1	0.5	499.5	6 minutes
1/26/97	2	10	490	1.25 minutes
1/26/97	3	15	485	45 seconds

Note:

1. See page 21 of the Methods section for a description of the Stock Solution.
2. See page 21 of the Methods section for a description of the Buffer Water.

Laboratory Experimental Setup and Procedures

The Laboratory experiments were conducted using the same test apparatus, bacteria preparation, and experimental procedures as discussed in the previously covered Control experiment section.

A total of twelve trials using *E. coli* were conducted on four separate dates. The concentration used and the length of time that each replicate was conducted is presented in Table 3. Ten experiments were conducted using *E. aerogenes* were conducted on two separate dates. The concentration used and the length of time that each replicate was conducted is presented in Table 4.

Table 3. *E. coli* Trial Characteristics

DATE	TRIAL NUMBER	STOCK SOLUTION[1] (mL)	BUFFER WATER[2] (mL)	TRIAL DURATION
7/20/96	1	15	485	1 minute
7/21/96	1	15	485	45 seconds
7/21/96	2	15	485	45 seconds
7/21/96	3	15	485	45 seconds
7/21/96	4	15	485	45 seconds
7/30/96	1	1	499	1 minute
7/30/96	2	2.5	497.5	2.75 minutes
7/30/96	3	5	495	2.5 minutes
7/30/96	4	10	490	1.25 minutes
7/30/96	5	15	485	45 seconds
8/1/96	1	0.25	499.75	9 minutes
8/1/96	2	0.50	499.5	6 minutes

Note:

1. See page 21 of the Methods section for a description of the Stock Solution.
2. See page 21 of the Methods section for a description of the Buffer Water.

Table 4. *E. aerogenes* Trial Characteristics

DATE	TRIAL NUMBER	STOCK SOLUTION[1] (mL)	BUFFER WATER[2] (mL)	TRIAL DURATION
8/3/96	1	15	485	45 seconds
8/3/96	2	10	490	1.75 minutes
8/3/96	3	2	498	2.75 minutes
8/3/96	4	1	499	4 minutes
8/3/96	5	0.5	499.5	6 minutes
8/5/96	1	15	485	45 seconds
8/5/96	2	10	490	1.75 minutes
8/5/96	3	2	498	2.75 minutes
8/5/96	4	1	499	4 minutes
8/5/96	5	0.5	499.5	6 minutes

Note:

1. See page 21 of the Methods section for a description of the Stock Solution.
2. See page 21 of the Methods section for a description of the Buffer Water.

Fish Culture Room Experimental Setup and Procedures

The Fish Culture Room at Olver Laboratories Inc., Blacksburg VA was used as the uncontrolled location for testing the UV chamber. Two types of experiments were performed at this location. The first tested the UV chamber's effectiveness in reducing the amount of bacteria found in the room and consisted of 15 trials. The second explored the effect that contact time had on the percent of bacteria that was killed. Five trials were conducted for this experiment.

The four topics that are discussed in the following section are:

- Site Description
- Apparatus
- Experimental Procedure
- Contact Time Experiment

Site Description

The Fish Culture Room is a 20 ft. x 10 ft. space with one door and no windows. *Pimephales promelas* (Fathead Minnow) are raised in 22 ten-gallon and four twenty-gallon aquariums located on three shelves. Each tank has a Whisper® filter and an air line that is attached to an air stone. Along the south wall are two 6 x 2 ft. water baths that are 2.5 inches deep. Spaced throughout the room are drain pipes that facilitate tank draining. These drain pipes are equipped with u-joints, however, evaporation results in occasional discharge of odors from these pipes. The room has one air conditioning vent located in the ceiling that is

part of the buildings central air conditioning system. The room is illuminated by 12, 40-watt fluorescent bulbs that operate for 16 hours/day on an automatic timer.

Apparatus

The UV chamber was setup with both end boxes on, as described in the "Ultraviolet Chamber Design" section then placed on a plastic cart that raised the chamber to three feet off of the ground. The UV light, chamber fan, and the Gast® Pumps for the Andersen Samplers were plugged into an extension cord. The Andersen Sampler at the outlet end of the UV chamber was situated so that the 18 inch plastic tube attached to it's inlet fit through the hole in the open-ended box covering the outlet. The Andersen Sampler rested on top of a five-gallon bucket that was placed on the floor next to the cart to make this possible. The Andersen Sampler at the inlet end was situated so that the 18 inch plastic tube attached to it's inlet cone was positioned adjacent to the inlet opening, but not in the path of the UV light.

Experimental Procedure

The conditions in the Fish Culture Room were kept constant during all trials (i.e. lighting, number of occupants, etc.). Every trial lasted 20 minutes. The UV chamber was operated in the same position in the room each time. All of the agar plates were incubated for 48 hours.

The following list is an example of the procedure used for the trials conducted in the Fish Culture Room:

1. The UV chamber and the Andersen samplers were arranged as previously discussed in the Apparatus section.
2. The two Andersen samplers were sterilized using the procedure mentioned previously in the Microbiological Methods. The sterility of the two Andersen Samplers was then tested by placing two agar plates (one in stage two and on in stage five) in the reassembled sampler for three minutes, with the pumps off. These two plates were then incubated along with the plates collected during the trial.
3. The fan and the UV light were turned on and a period of three minutes elapsed before the next trial began. During this three minute period, the relative humidity and temperature were measured. All six stages of both Andersen samplers were then loaded with agar plates and the samplers were reassembled.
4. The Andersen samplers were simultaneously turned on for 20 minutes. Both samplers were then turned off simultaneously, the plates were removed and the lids were reattached.

5. Steps 2 - 4 were repeated until all trials for that day were completed.
6. All of the agar plates were then placed in the incubator at the same time.

Contact Time Experiment

This experiment was undertaken in order to determine the optimal contact time for this UV chamber. Different air flows were tested by attaching a Powerstat® Variable Autotransformer, manufactured by The Superior Electric Company, Bristol, Conn., to the fan. The voltage settings that were tested included 80, 90, 100, 110, and 120 volts. The air flow that corresponded to each of these settings was measured after the testing was completed.

Ultraviolet Chamber Flow Measurements

The type of fan that was installed in the UV chamber was designed for cooling electronic equipment. Since the chamber installation was different from what the fan was designed for and to ensure accurate interpretation of the data, flow measurements were required. A Keuffel & Esser Co., New York, type Vane Anemometer was chosen for this purpose. This anemometer was capable of measuring very low air flows. The instrument was borrowed from the Instrument Room located in the Mechanical Engineering Department at Virginia Polytechnic Institute and State University, Blacksburg. Three flow measurements

were taken for each voltage setting that was tested including the normal operating condition - 120 volts. The average of these three measurements was then used to calculate the contact time and volume of air that was moved through the chamber. The bench sheets and sample calculations for the flow measurements can be found in Appendix D.

Data Analysis

Collection Efficiency Comparison of the two Andersen Samplers

Prior to the commencement of testing, the two Andersen® cascade impactors were tested to ensure that they would collect the same amount of particles. The two samplers were disinfected with isopropyl alcohol and allowed to dry, then were loaded with agar plates and reassembled. By using a power strip both samplers were turned on at the exact same time and run for a duration of 20 minutes. This procedure was repeated four more times after which the plates were labeled and incubated at the same time, temperature, and for the same duration. The number of colonies formed were counted and recorded. Using the statistical software contained in Word Perfect's Quatro Pro, the results from all five trials were compared and a t-test was performed. The statistical results from the comparison testing are in Appendix E.

Laboratory and Fish Culture Room Data

The data from both the Laboratory and Fish Culture Room trials were analyzed using the statistical software contained in Word Perfect's Quatro Pro. A t-test was performed on the bacteria counts from each trial. The pre-UV counts were compared to the post-UV counts to see if the percent of bacteria killed by the UV light was significant. The percent of bacteria killed during each trial was then compared to the density of bacteria and presented in graphical form. A linear regression was performed on the data and an R^2 value was generated. The linear regression was then plotted on each graph.

CHAPTER IV. RESULTS

This chapter contains the results from the Control, Laboratory and Fish Culture Room Experiments. The Laboratory and Control data includes both the *E. coli* and *E. aerogenes* trials. The Fish Culture Room data include the results from trials conducted on four trial dates. The first three dates were exact replicates involving the same testing parameters, while the fourth date explored the effect that contact time has on the percent of bacteria killed. The bench sheets from the Control trials are contained in Appendix F, bench sheets from the Laboratory trials are contained in Appendix G, and bench sheets from the Fish Culture Room trials are contained in Appendix H.

Control Experiments

Tables 5 and 6 show the Percent Kill for the 6 trials conducted with *E. coli* and *E. aerogenes*, respectively. The Percent Kill was determined by subtracting the total number of colony forming units (CFU) collected after the aerosolized bacteria passed through the UV light chamber from the total number of CFU's collected before the aerosolized bacteria entered the chamber, and dividing that difference by the total number of CFU's collected before the aerosolized bacteria entered the UV light chamber. The CFU density was determined by dividing the total number of CFU's collected before the aerosolized bacteria entered the UV light chamber by the amount of time that the trial lasted. Since the Anderson Samplers were calibrated to pull one ACFM the number of minutes that the trial lasted directly relates to the volume of aerosolized bacteria that was pulled through the sampler. The percent kill shifted between negative and positive results, this was more

evident in the *E. coli* results as compared to the *E. aerogenes* results. The temperature for each day remained stable throughout the trials as did the relative humidity.

Table 5. Results from Control Trials with *E. coli*

DATE	TRIAL NUMBER	DENSITY (CFU/m^3)	PERCENT KILL	TEMPERATURE (ºF)	RELATIVE HUMIDITY (%)
1/18/97	1	1	-17	68	64
1/18/97	2	153	3	68	64
1/18/97	3	608	-2	68	64
1/19/97	1	0.66	-75	68	70
1/19/97	2	141	2	68	70
1/19/97	3	564	4	68	70

Table 6. Results from Control Trials with *E. aerogenes*

DATE	TRIAL NUMBER	DENSITY (CFU/m^3)	PERCENT KILL	TEMPERATURE (ºF)	RELATIVE HUMIDITY (%)
1/25/97	1	52	-2.6	70	72
1/25/97	2	185	1.3	70	72
1/25/97	3	614	2	70	72
1/26/97	1	76	0.9	70	68
1/26/97	2	208	1.9	70	68
1/26/97	3	627	1.6	70	68

Laboratory Experiments

Table 7 shows the Percent Kill for the 12 trials conducted with *E. coli*. The *E. coli* concentrations for the twelve trials ranged from 0.66 to 833 CFU/m^3. The percent kill for all 12 trials was 100%. The temperatures remained stable throughout the trials conducted on each day. A 3º F difference occurred between the four trial dates, with the higher temperature of 74º F occurring on the first date (7/20/96). The relative humidity remained stable throughout the trials conducted on each day, with it being higher on the third and fourth days.

Table 7. Results from Laboratory Trials with *E. coli*

DATE	TRIAL NUMBER	DENSITY (CFU/m^3)	PERCENT KILL	TEMPERATURE (ºF)	RELATIVE HUMIDITY (%)
7/20/96	1	455	100	74	52
7/21/96	1	88	100	71	58
7/21/9	2	208	100	71	58
7/21/96	3	221	100	71	58
7/21/96	4	111	100	71	58
7/30/96	1	29	100	70	72
7/30/96	2	63	100	70	72
7/30/96	3	177	100	70	72
7/30/96	4	165	100	70	72
7/30/96	5	833	100	70	72
8/1/96	1	0.66	100	70	72
8/1/96	2	0.83	100	70	72

Table 8 shows the percent kill for the ten trials conducted with *E. aerogenes*. The concentration of *E. aerogenes* for the these trials ranged from 65 to 600 CFU/m³. A percent kill of 97% was the highest obtained, representing the trial with the initial *E. aerogenes* concentration of 600 CFU/m³ (the highest concentration of *E. aerogenes*). The lowest percent kill corresponded to the *E. aerogenes* concentration of 65 CFU/m³ (the lowest concentration of *E. aerogenes*). The temperature and relative humidity remained stable throughout each trial date with a 2º F temperature and 9% relative humidity difference between the two dates.

Figure 3 illustrates the data presented in Table 8. The percent kill is compared to the density of *E. aerogenes* in each trial. The normalized density was determined by dividing the density that was measured for each trial by the highest density that was measured during the ten trials (in this case 600 CFU/m³ was the highest measured, corresponding to Trial 1 on 8/5/96). A linear regression was used in the figure to show the correlation of the two variables, and was generated by using the Quatro Pro Software contained in Novell's Perfect Office®. This same software was used for all of the linear regression data used on the figures in this section.

Table 8. Results from Laboratory Trials with *E. aerogenes*

DATE	TRIAL NUMBER	DENSITY (CFU/m^3)	PERCENT KILL	TEMPERATURE (^oF)	RELATIVE HUMIDITY (%)
8/3/96	1	532	96	70	62
8/3/96	2	209	92	70	68
8/3/96	3	123	90	70	68
8/3/96	4	85	86	70	68
8/3/96	5	65	82	70	68
8/5/96	1	600	97	68	59
8/5/96	2	310	94	68	59
8/5/96	3	152	91	68	59
8/5/96	4	93	88	68	59
8/5/96	5	74	84	68	59

Figure 3 *E. aerogenes* Trials - Density vs. Percent Kill Page 42 (42.jpg 45.4 KB)

Fish Culture Room Experiments

Table 9 shows the percent kill, density of bacteria in the air stream prior to entering the UV light chamber, temperature and relative humidity for the three dates that trials were conducted in the Fish Culture Room. The five trials for each day were run consecutively, with only a five minute break between trials for disinfection of the sampler. The 15 trials each lasted 20 minutes. The density of bacteria measured during the five trials varied from as little as 0.15 CFU/m^3 during the trials on 7/17/96 to as much as 2.1 CFU/m^3 during the trials on 7/29/96. Both the temperature and relative humidity remained constant during all five trials conducted each day. The relative humidity varied by two percent throughout the three days that trials were conducted. The temperature was the same for the first and third trial dates and only two degrees higher during the second trial date.

Figures 4, 5, and 6 show the results from June 19, July 17 and 29, respectively. The percent kill is compared to the normalized density in all three graphs. A linear regression shows the correlation between the two variables. Appendix D contains the statistical data for the linear regression that were plotted on the graphs. Notice for all three dates that the percent kill increased as the density increased. The normalized density was used on the graphs in order to put all of the results from the three sets of Fish Culture trials on the same scale for easy comparison. The density that was calculated for each trial and presented in Table 3 was used to calculate the normalized density.

Table 9. Fish Culture Room Experiments

Trial Date	Trial Number	Density (CFU/m^3)	Percent Kill	Temperature (^oF)	Relative Humidity (%)
6/19/96	1	2.50	36	75	66
	2	3.05	56	75	66
	3	2.15	28	75	66
	4	2.55	41	75	66
	5	3.00	63	75	66
7/17/96	1	1.70	44	77	68
	2	1.55	21	77	68
	3	1.60	29	77	68
	4	1.65	42	77	68
	5	1.60	31	77	68
7/29/96	1	3.90	99	75	67
	2	2.40	42	75	67
	3	2.75	84	75	67
	4	1.55	71	75	67
	5	1.80	75	75	67

Figure 4 Fish Culture Room - June 19, 1996 Page 45 (45.jpg 42.2 KB)

Figure 6 Fish Culture Room - July 29, 1996 Page 46 (46.jpg 42.6 KB)

Figure 6 Fish Culture Room - July 29, 1996 Page 47 (47.jpg 40.4 KB)

Figure 7 shows the accumulated data from all the fifteen trials represented in Figures 4, 5, and 6. The percent kill and normalized density used in this figure were calculated in the same manner as they were calculated for the three figures that were used to develop them (Figures 4, 5, and 6).

Contact Time vs. Percent Kill

The results of the experiment on 8/2/96 dealing with the influence that contact time has on the percent kill are contained in Figure 8. This graph shows that the percent kill increased as the contact time increased until it peaked at 92%. The 0.265 second contact time represents the normal operating setting of 120 volts.

Figure 7 Fish Culture Room - All Three Trial Dates Page 49 (49.jpg 43.2 KB)

Figure 8 Contact Time vs. Percent Kill Page 50 (50.jpg 35.4 KB)

CHAPTER V. DISCUSSION

Effectiveness

The UV chamber's success in eradicating bacteria cannot be precisely quantified based on the results from the two experiments. While the chamber was 90 and 100% effective against the *E. aerogenes* and *E. coli*, respectively, it was only 51% effective against the bacteria found in the Fish Culture Room. The possible explanation for this discrepancy is discussed in the following sections. Despite the fact that the UV chamber was very effective in the laboratory setting against the pure cultures of bacteria, it has to be effective in the "real world" settings like the Fish Culture Room. Based on Figure 8, increasing the contact time to around 0.33 seconds may increase the percent kill to a satisfactory level. The position and number of bulbs needs to be considered in addition to the contact time.

The Phillips Lighting Company's Customer Service Center recommended positioning the bulb perpendicular to the air flow and adding another bulb to decrease the distance that the bacteria could possibly be from the UV source at any time while in the chamber. An abstract printed in the TB Weekly (27) stated that Scarpino *et al.* (1995) designed a UV chamber that was 99% effective in killing *E. coli*. That chamber utilized four UV bulbs (the type and position of the bulbs was not included in the abstract) and could handle a flow rate of 275 cfm.

Under normal operating conditions, 120 volts, the UV chamber handles a flow of 158 cfm, corresponding to an additional nine equivalent air changes per hour for a room 10 x 12

x 9 feet. If the air flow were manipulated so that the contact time could be increased to around 0.332 seconds, then the UV chamber would have an air flow of 126 cfm, corresponding to an additional seven equivalent air changes per hour for the same size room.

UV Light Chamber Design

The design of the UV chamber succeeded in addressing the concerns related to exposure to the UV radiation. The open-ended boxes that were attached to the ends of the chamber, along with the black lining of those boxes kept the release of UV light to a minimum. The need to facilitate sampling prevented further steps that would have reduced the amount of UV light that escaped the chamber. The entrance and exit areas of the chamber need to be moved in order to reduce the chance of reintraining the exhaust back into the chamber, which would reduce the volume of air being treated by the chamber. The manufacturer of the UV bulb, Phillips Lighting Company, states in their Lamp Specification and Application Guide that this bulb produces negligible amounts of ozone. This was a concern during the designing of the chamber due to the problems associated with ozone in indoor environments. The temperature output of the UV chamber was also explored in addition to the ozone production by measuring the temperature difference between the intake and exhaust of the chamber during each trial. The temperature of the exhaust was only 1 - 2 ^{o}F higher than the intake air stream.

Laboratory Experiment

The results from the laboratory experiments shows that the UV chamber's ability to kill bacteria varies depending on the bacteria. The two bacteria were tested under the exact same conditions and by the same methods. The chamber was very effective against the *E. coli* bacteria, exhibiting nothing less than a 100% kill for all 12 concentrations. A wide variety of concentrations were tested in an attempt to achieve a kill of less than 100%. These concentrations ranged from as little as 0.66 CFU/m^3 to as much as 833 CFU/m^3. Whereas the experiments with *E. coli* did not achieve a kill of less than 100%, the experiments with *E. aerogenes* did not achieve a kill rate of 100%. The concentrations of *E. aerogenes* tested ranged from as little as 65 CFU/m^3 to as much as 600 CFU/m^3. The highest and lowest concentrations of *E. aerogenes* corresponded to the highest and lowest percentage of bacteria killed, respectively. These results showed that as the density increased, the percent of bacteria killed by the UV chamber increased, a trend also seen in the Fish Culture Room data. The possible explanations for this trend will be discussed with the Fish Culture Room data. The reason that the UV affected the *E. coli* differently than the E. aerogenes may be found in the physical characteristics of the bacteria. As mentioned in the Literature Review, the UV affects the bacteria's DNA, specifically the thymine bases. With this in mind, the size of the bacteria cell and the amount of thymine in the DNA sequence may be the explanation for the different effect that was seen.

Control Experiments

The results from the control experiments showed that the ultraviolet chamber does not have an effect on the bacteria passing through it. Neither a beneficial or deleterious effect was evident from the twelve trials that were performed. The percent kills for the individual trials shown in Table 5 varied, however, the small number of colonies that were tested during those trials that exhibited the largest variation led to the seemingly large percent kills that were observed.

Fish Culture Room Experiment

The trials from the Fish Culture Room show that as the density of bacteria increased the percent of bacteria that was killed increased. A linear regression was performed on the data from each trial day and the resulting line was displayed on the graph for that day (Tables 2-4). The R^2 values for the linear regressions performed on each of the five trial dates were:

Table 10. Statistical Results for Fish Culture Room Trials

Trial Date	R^2 Value
6-19-96	0.93
7-17-96	0.94
7-29-96	0.31
7-29-96**	0.99

**Data from Trial 2 on 7-29-96 not included.

Individually the data from each trial date shows a relatively good relationship between the density of bacteria and the percent kill. Five trials were performed on each date with the duration of each trial being 20 minutes. An R^2 values less than 1.00 may be attributed to the

types and relative proportions of bacteria that were in the air stream. Based on the Laboratory Experiment trials, and the fact that a number of different types of bacteria are likely to be found in the Fish Culture Room, it is possible that a change in the type and relative proportion of bacteria caused changes in the percent of bacteria that was killed.

The reason that the R^2 value for the 7/29/96 trials was performed twice was due to one aberrant point that was obtained on that date. One point varied significantly from the other four points indicating that this point may have been caused by some error during either sampling or enumeration. Without this point the R^2 value went form 0.31 to 0.99. The modified graph is shown in Figure 9.

Ideally a larger data set for either the Laboratory or Fish Culture Experiments would lead to more accurate statistical results. Due to the long duration of each trial and the time required between each trial for disinfection of the samplers, it was decided that any additional trials would increase the chance that one or more conditions of the trial would change. The five trials performed on each of the three days took about two hours to complete; additional trials would require an extra 25 minutes.

Contact Time vs. Kill Rate Experiment

A screening test on 8/3/96 was conducted to see what the optimal contact time would be for this specific UV Chambers. The optimal contact time would be the time period that elicits the highest bacteria kill. Figure 8 shows that as the contact time was increased from 0.272 seconds to 0.415 seconds, the percent of bacteria that was killed increased. That rise

Figure 9 Fish Culture Room - July 29th without 3rd Trial Page 56 (56.jpg 44.8 KB)

in the Percent Kill peaked around 0.332 seconds and then declined as the contact time approached 0.415 seconds. These data indicate that the optimal contact time for this specific UV chamber would be around 0.332 seconds. Based on the laboratory experiments it would be unwise to assume that this contact time would be optimal for all settings and building environments. The type and ratio of bacteria present will play a role in determining the optimal contact time for individual locations. By increasing the size of the area that the air stream comes into contact with the UV light while maintaining the velocity would achieve both the lower contact time that is needed and maintain the volume of air that is treated per minute. It is important to be able to treat as much air per minute as possible while effectively reducing the amount of bacteria in the air. This would allow the UV unit to be competitive with the other air cleaning devices currently on the market.

Stressed Organism Collection

Additional testing was proposed that would allow for the collection and enumeration of the number and extent of organisms that would appear to be killed by the ultraviolet radiation when in reality those organisms may only be inactivated. These organisms are properly referred to as "stressed organisms". They do not show any growth on normal media, during the typical incubation period. However, upon discussing this proposal with Dr. Robert Benoit, Virginia Polytechnic Institute and State University, it was decided not to pursue this line of testing (28). Dr. Benoit expressed his reservations regarding the procedures that are currently being used to collect stressed organisms. In summary, he felt that this line of testing would not be beneficial and did not recommend including it due to the collection methods and

types of media that were used in the tests that were conducted in this thesis. The collection methods and type of media that were used in this thesis were, in his opinion, beneficial in increasing the chance of collecting and accounting for stressed organisms.

CHAPTER VI. CONCLUSIONS AND RECOMMENDATIONS

1. A lightweight, portable UV disinfection unit has been developed that reduces airborne bacteria.
2. As the density of *E. aerogenes* increased the percent killed by the UV radiation increased.
3. The percent of bacteria that can be killed by the UV chamber is dependent on the types and relative percentage of bacteria present in the air stream.
4. The UV light chamber was not as effective in killing bacteria in an actual indoor environment as it was in killing a homogeneous concentration of bacteria.

Further testing should be performed to test the UV chamber's effect on a wide range of bacteria and fungi. Since a diverse and lengthy list of bacteria and fungi affect the quality of indoor air, testing a larger number of these would further verify the chamber as a legitimate control device. A number of modifications to the design of the chamber need to be explored. The contact time for the unit must be adjusted so that the optimal percent kill can be obtained. The number of lights and position of those lights should be adjusted so that the percent of bacteria that can be killed can be increased along with the flow. This would allow the chamber to treat more room air and kill more of the bacteria and fungi in that room at a faster rate.

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APPENDIX A

PSEUDOMONAS TRIALS; DESCRIPTION, RESULTS AND DISCUSSION

The trials involving *Pseudomonas* were not included in the body of the thesis due to the problems that arose during testing. The aerosolizing phase failed to produce enough *Pseudomonas* to conduct a test of the UV chamber.

The *Pseudomonas* bacterial disk that was used to start the bacteria culture was supplied by DIFCO. The disk was placed in 100 mL of Tryptic Soy Broth, supplied by BBL. The disk was incubated in this media at 35º C ± 0.2 for 48 hours and then was diluted with Buffer water made according to Section 9050C in Standard Methods. Following the mixing of the bacteria with the Buffer water the mixture was placed in the aerosol chamber previously described in the Methods and Materials section. The *Pseudomonas* was then aerosolized following the same procedure that was executed for both *E. coli* and *E. aerogenes.* Six different dilutions were tested on three different dates. Both Standard Methods Plate Count Agar and Tryptic Soy Agar were used to collect the bacteria. The results were not favorable. Only two of the 12 trials conducted showed any colonies on the pre-UV plates. These two trials did not contain enough colonies for the results to be defendable. The viability of the bacteria solution was tested by directly inoculating agar plates with the initial bacteria solution. *Pseudomonas* colonies grew on these plates. Since the sterility of the media was checked along with the viability of the bacteria, aerosolization was considered to be the source of the problems. Furthermore, since the aerosolizing chamber worked for the *E. coli* and *E. aerogenes* the problem was not with the aerosolizing chamber but with the bacteria's ability to be aerosolized.

APPENDIX B

BENCH SHEETS FOR THE SPRAGUE DRY TEST METER CALIBRATION

Appendix B Page 66 (66.jpg 95.7 KB)

Appendix B Page 67 (67.jpg 91.5 KB)

Appendix B Page 68 (68.jpg 93.8 KB)

Appendix B Page 69 (69.jpg 96.8 KB)

APPENDIX C

STATISTICAL DATA FOR THE ANDERSON SAMPLER COMPARISON TESTS

Appendix C Page 71 (71.jpg 34.3 KB)

APPENDIX D

FLOW MEASUREMENT SAMPLE CALCULATION, BENCH SHEETS, AND DATA

Sample Calculation

Chamber Length : 24 inches (2 feet)

Chamber Diameter : 8 inches (0.6667 feet)

Area of Chamber (cylinder) : 0.698 ft^3

Area of Chamber opening : 0.349 ft^2

Flow measurements at 80 volts : 286, 291, and 293 feet/minute

Average of the three measurements = 290 feet/minute

290 feet/minute x 0.349 ft^2 = 101 cfm

Contact time :

0.698 ft^3 ÷ 101 cfm = 0.00691 minutes

0.00691 minutes = 0.415 seconds

Flow Measurement Data

80 Volts

1. 286 feet/minute
2. 291 feet/minute
3. 293 feet/minute **Average = 290 feet/minute**

90 Volts

1. 360 feet/minute
2. 363 feet/minute
3. 363 feet/minute **Average = 362 feet/minute**

100 Volts

1. 411 feet/minute
2. 413 feet/minute
3. 412 feet/minute **Average = 412 feet/minute**

110 Volts

1. 437 feet/minute
2. 441 feet/minute
3. 442 feet/minute **Average = 440 feet/minute**

120 Volts

1. 447 feet/minute
2. 451 feet/minute
3. 452 feet/minute **Average = 450 feet/minute**

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Appendix D Page 76 (76.jpg 58.8 KB)

APPENDIX E

STATISTICAL DATA FOR FISH CULTURE TRIALS

Appendix E Page 78 (78.jpg 35.3 KB)

APPENDIX F

CONTROL EXPERIMENT BENCH SHEETS

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Appendix F Page 81 (81jpg 61.5 KB)

Appendix F Page 82 (82.jpg 62.5 KB)

Appendix F Page 83 (83.jpg 64 KB)

Appendix F Page 84 (84.jpg 64.1 KB)

Appendix F Page 85 (85.jpg 65.0 KB)

APPENDIX G

LABORATORY EXPERIMENT BENCH SHEETS:

ESCHERICHIA COLI AND *ENTEROBACTER AEROGENES*

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Appendix G Page 90 (90.jpg 58.4 KB)

Appendix G Page 91 (91.jpg 59.8 KB)

Appendix G Page 92 (92.jpg 58.2 KB)

Appendix G Page 93 (93.jpg 59.7 KB)

Appendix G Page 94 (94.jpg 60.1 KB)

Appendix G Page 95 (95.jpg 60.5 KB)

Appendix G Page 96 (96.jpg 59.8 KB)

Appendix G Page 97 (97.jpg 60.2 KB)

APPENDIX H

FISH CULTURE ROOM EXPERIMENT BENCH SHEETS

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Appendix H Page 101 (101.jpg 58.2 KB)

Appendix H Page 102 (102.jpg 57.6 KB)

Appendix H Page 103 (103.jpg 58.6 KB)

Appendix H Page 104 (104.jpg 57.4 KB)

Appendix H Page 105 (105.jpg 58.4 KB)

Appendix H Page 106 (106.jpg 55.2 KB)

VITA

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The author was born in Charleston, South Carolina on March 25, 1969. He graduated from Western Wayne High School in Pennsylvania in June, 1987. He attended Roanoke College from 1987-91 and graduated with a Bachelor of Science degree. He attended Virginia Polytechnic Institute and State University from 1993-97 where he received a Master's degree in Environmental Science and Engineering. He is currently working for a environmental laboratory and consulting firm, is married and has two children.