T.C.
FATIH UNIVERSITY
INSTITUTE OF BIOMEDICAL ENGINEERING

EVOLUTION OF ULTRA VIOLET LIGHT SUCCESS AS A DISINFECTION TECHNIQUE

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MSc THESIS
BIOMEDICAL ENGINEERING PROGRAMME

İSTANBUL, FEBRUARY / 2016

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ASSIST. PROF. DR. ŞÜKRÜ OKKESİM

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FATİH ÜNİVESİTESİ
BİYOMEDİKAL MÜHENDİSLİK ENSTİTÜSÜ

BİR DEZENFEKSİYON YÖNTEMİ OLARAK ULTRA VİOLE IŞIĞIN DEĞERLENDİRİLMESİ

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YÜKSEK LİSANS TEZİ
BİYOMEDİKAL MÜHENDİSLİĞİ PROGRAMI

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İSTANBUL, ŞUBAT / 2016

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FATIH UNIVERSITY

INSTITUTE OF BIOMEDICAL ENGINEERING

İsmail Aldemir, a **MSc** student of Fatih University **Institute of Biomedical Engineering** student ID **520112014**, successfully defended the **thesis** entitled " **Evolution of Ultra Violet Light Success As A Disinfection Technique**", which he/she prepared after fulfilling the requirements specified in the associated legislations, before the jury whose signatures are below.

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This thesis is dedicated to my parents and my wife who have supported me all the way since the beginning of my studies,

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11 December 2015 İsmail Aldemir

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LIST OF SYMBOLS

C	Correlation index
λi	Lambda factor
Ω	Omega
ψ	Positive number
Δ	Delta function
δ	Distance

ABBREVIATIONS

C	: Degree Celsius
CDC	: Centers for Disease Control and Prevention
cm	: Centimeter
h	: Height
m3	: Cubic Meter
ml	: Milliliter
mm	: Millimeter
TSA	: Triptych Soy Agar
TSB	: Triptych Soy Broth
Uv	: Ultra-violet

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SUMMARY

EVOLUTION OF ULTRA VIOLET LIGHT SUCCESS AS A DISINFECTION TECHNIQUE

İsmail ALDEMİR

Biomedical Engineering Programme

MSc Thesis

Assist. Prof. Dr. Şükrü Okkesim

The main source of electromagnetic radiation is the sun. It has a continous effect to the world. Electromagnetic radiation consists of gama rays, X rays, UV rays, infrared rays, visible rays, microwaves and radiowaves. Electromagnetic waves are harmful to the environment, especially to microoganisms. If the rays get in touch with the organisms, the energy gets absorbed by the cellular elements, this leads to damage to the cell or to the death of the cell. This especially occurs within short wave lenghts, high energy owned electromagnetic rays which are gama-, X-, UV Rays. UV rays dont need high temperatures to kill microorganisms, thats why they are getting used as a disinfectant for a long time disinfection and sterilization techniques that are used today occasionally includes physical and chemical methods, both techniques have some advantages and disadvantages according to the materials that are used. Especially new methods are under-research in environment disinfection due to issues that these methods may have, One of these methods is under-research is disinfection with Ultra-Violet light method. There are many usage areas of Ultra-Violet lamps for its disinfection functions, the epidemic and efficient usage of UV lamps is seen especially in air and ground disinfections, the disinfections of operation rooms, labs and biological security cabins. Furthermore, in

common places of people such as: kindergarten, cafes, gyms, markets and closed spaces like hospital rooms; UV lamps could be used to prevent illnesses that spread via air by decreasing pathogenic organisms. When coming to water disinfection the UV lamps method started to be used occasionally in obtaining drinking water and treatment facilities, another usage occurs in material disinfection such as medical equipment and particularly in the disinfection of materials which has no heat and moisture resistance. In this dissertation paper it has researched advantages and disadvantages of other sterilization techniques and, if efficient usage of UV lamps is possible or not. The data obtained was asserted in arguments and conclusions passage and it has been concluded the usage of UV light is an efficient method.

Keywords: Ultra violet, disinfection, sterilization, p*seudomonas aeruginosa*.

FATIH UNIVERSITY - INSTITUTE OF BIOMEDICAL ENGINEERING

ÖZET

BİR DEZENFEKSİYON YÖNTEMİ OLARAK ULTRA VİOLE IŞIĞIN DEĞERLENDİRİLMESİ

İsmail ALDEMİR

Biyomedikal Mühendisliği Programı
Yüksek Lisans Tezi

Danışman: Yrd. Doc. Şükrü Okkesim

Elektromanyetik radyasyonun başlıca kaynağı güneştir ve dünya üzerinde sürekli etki oluşturmaktadır. Elektromanyetik radyasyon; gama ışınları, X ışınları, ultraviyole (UV) ışınları, görünür ışık, infrared ışınlar, mikrodalgalar ve radyo dalgalarından oluşur. Elektromanyetik radyasyonun canlılara, özelliklede mikroorganizmalara zararı büyüktür. Bu ışınlar organizmaya temas ettiğinde, enerji hücresel elemanlarca absorbe edildiği için hücre hasarına veya hücrenin ölümüne neden olur. Bu özellikle kısa dalga boylu, yüksek enerjili elektromanyetik radyasyon olan gama ışınları, X ışınları ve UV ışık için geçerlidir. UV ışınları mikroorganizmayı öldürmek için ısıya ihtiyaç duymamaları nedeniyle uzun zamandır bir dezenfektan olarak kullanılmaktadır. Günümüzde sıklıkla kullanılan dezenfeksiyon ve sterilizasyon teknikleri fiziksel ve kimyasal metotlar içermektedir. Her iki teknik uygulanan malzemeye göre avantajlara ve dezavantajlara sahiptir. Özellikle ortam dezenfeksiyonunda bu iki yöntemin taşıdığı problemler nedeniyle yeni yöntemler araştırılmaktadır. Araştırılan bu yöntemlerden birisi de ultraviyole ışık ile dezenfeksiyondur.

Ultraviyole Lambalarının birçok dezenfeksiyon amaçlı kullanım alanları vardır. UV ışığın hava ve yüzey dezenfeksiyonu özelliği, ameliyathanelerde, laboratuarlar ve biyolojik güvenlik kabinlerinde etkilidir. Ayrıca, insanların ortak kullanım alanları,

kalabalık olarak bulunduğu kreşler, kafeteryalar, jimnastik salonları, marketler, hastane odaları gibi kapalı yerlerde, havada bulunan patojen mikroorganizmaların sayısını azaltarak hava yolu ile bulaşan hastalıkların yayılımını engellemek için kullanılabilirler. Su dezenfeksiyonunda ise günümüzde yaygın olarak, içme suyu elde etmede ve arıtma tesislerinde kullanılmaya başlanmıştır. Bir diğer kullanımı ise alet dezenfeksiyonu olup genelde tıbbi cihazlarda ve özellikle ısıya ve neme dayanıksız materyalin dezenfeksiyonunda kullanılabilir. Bu tez çalışmasında diğer sterilizasyon tekniklerinin avantaj ve dezavantajları araştırılıp, ultraviole ışınların ortam dezenfeksiyonunda verimli kullanılıp kullanılamayacağı araştırılmıştır. Elde edilen veriler sonuç ve tartışmalar kısmında değerlendirilmiş ve ultraviole ışınla ortam dezenfeksiyonunun verimli bir yöntem olduğu sonucuna varılmıştır.

Anahtar kelimeler: Ultraviole, dezenfeksiyon, sterilizasyonon, *Pseudomonas aeruginosa*

CHAPTER 1

INTRODUCTION

1.1 Purpose of the Thesis

Ultraviolet light (UV) is an invisible, electromagnetic radiation with a wavelength from 400 nm to 10 nm. UV allows us to disinfect or sterilizise the place that it hits. The procedure is mainly like this: The radiation goes through the cell membrane of the bacteria, passes through the cell body, change the DNA structure and kill the microorganism [1, 2].

The purpose of this thesis is to evaluation of the UV technology for environmental disinfection. Nowadays, one of the most important problems of the place that people live is infectious bacteria. There are many usage areas of Ultra-Violet lamps for its disinfection functions, the epidemic and efficient usage of UV lamps is seen especially in air and ground disinfections, the disinfections of operation rooms, labs and biological security cabins. Furthermore, in common places of people such as: kindergarten, cafes, gyms, markets and closed spaces like hospital rooms, UV lamps could be used to prevent illnesses that spread via air by decreasing pathogenic organisms [3-6] and UV lamps method started to be used occasionally in obtaining drinking water and treatment facilities [7-10].

In order to diminish the bacteria from the environment, between other disinfection techniques[11], we think that 254 nm ultra violet light is a good disinfector [2].

1.2 Arrangement of the Thesis

At the beginning of the investigation we starded with research the appropriate UV light to obtain maximum effectiveness.

After that we need a light system for making our experiments. The devices like UV light, the protector box, the electronic part (UV light driver) bought from reliable manufacturers to obtain objective results.

In this research Pseudomonas aeruginosa was focused. In appropriate laboratory conditions the bacteria cultured and wait for proliferation.

In the experimental stage 20 bacteria plates were used (10 for UV experiments,10 for control groups) for statistically acceptable results.

At the last part the results are compared and discussed.

CHAPTER 2

BACKGROUND INFORMATION

2.1 Literature Survey

Sterilization is the process of removing (killing) all alive microorganisms including bacterial spors. If we take a look at the historical background of the process of sterilization we will see that the roots are very deep. The Egyptians used antiseptics such as pitch or tar. They used it to embalm their bodies. The searchings of Robert Koch resultet in developing the first nonpressure flowing steam sterilizer. The American physician Earle Spaulding, showed how an object should be disinfected or sterilized depending on the object's intended use (1968). In 1994 William Rutala worked with the CDC to characterze an ideal method of sterilization [12, 13]. Within the century of innovation and science the technology of sterilization is improoving. Nowadays there are 2 common methods.

These are the physical and the chemical method.

2.2 Physical Methods of Sterilization

Sunlight: It is responsible for spontaneous sterilization in natural conditions.

By killin bacteria suspended in water, sunlight provides naturat method of disinfection of water bodies such as tanks and lakes[14].

Heat: Heat acts by oxidative effects as well as denaturation and coagulation of proteins.

Action of Heat: Dry functions by protein denaturation, oxidative damage and toxic effects of elevated levels electrolytes.

Dry Heat: Causes denaturation of protein and oxidative damage

• Red Heat

• Flaming

• Incernation

• Hot Air oven

• Infra red rays

Moist heat has a bigger effect in contrast to dry heat;

Moist Heat:

Moist heat has a bigger effect in contrast to dry heat leads coagulation and denaturation of proteins.

At temperature below 100°C:

Pasteurisation: Tis procedure is employed in food and dairy industry.

Vaccine bath: Bacteria in a vaccine preperation occured in a water bath at 60 c for 1 hour. Only vegetative bacteria are killed.

Serum bath: 56 c heated for 1 hour, spores survive.

Inspissation: The process depends on germination of spores in between ispissation. On the first day the vegetative bacteria would die. Spores are geminated by next day and then they are killed.

At temperature 100°C:

Boiling: Boiling water kills most vegetative bacteria and viruses immediately.

Steam: Instead of boiling water, the bacterias are subjected to free steam at 100 c

At temperature above 100°C:

Autoclave: Sterilization can be effectively accomplished by a tempreture above 100c using the autoclave.

Filtration: Filtration doesn’t destroy microbes, instead of this it seperates them. It’s generally used at microbiological labs

2.3 Chemical Methods of Sterilization:

Disinfectants are chemicals those are used to get rid of hazardous bacteria from a surface. Those chemicals that can be safely applied over skin and mucus membranes are called antiseptics.

The chemicals Methods are divided into 2:

These are liquids and gaseous

Ethylene oxide: Ethiylen oxide is the mos commonly used sterilization gas vapor. But its usage is limited because of its hazardous features. It has limited use, because of its harmful properties.

Formaldehyde gas: Formaldehyde gas is another harmful sterilization gas. Since it is carcinogenic, its usage is limited primarily on HEPA filters.

H_20_2: Hydrogen peroxide is an effective sterilization gas with less harmfull properties comparing to ethylene oxide and formaldehyde gas. Its working principle is based on its oxidation power [15].

Peracetic acid: Peracetic acide is obtained from the reaction of hydrogen peroxide and acetic acide. It is colorless with a pH 2,8. Produced by the reaction of hydrogen peroxide and acetic acid. Glutaraldehyde: a colourless, pungent liquid produced industrially by the oxidation of cyclopentene.

Radiation: There are two types of radiation which are ionizing and non-ionizing.

Ionizing rays: Ionizing raysa are two types electromagnetic and particulate rays.

Non-ionizing rays: wavelengt longer than the visible light are non-ionizing rays.

Ultra violet light is a non-ionizing ray.

2.4 What is UV radiation?

UV is an electromagnetic radiation which is thinner than visible light but it is thicker than X light as it has longer wave lengths, (Approximately 10-400 nm). UV radiation can split into two stages according to its wave lengths, one being far UV (Extreme-UV 10-200nm) and the other being close UV (near-UV, 200-380nm). Close UV is split into 3 stages as it affects human health and the environment, to be regarded by others.

The 3 stages are:[9]

• UVA (long UV, long wave UV, black light; 315-400 nm),

• UVB (medium UV, medium wave UV; 280-315 nm)

• UVC (short UV, shortwave UV, germicidal UV; 200-280 nm)

UV can kill all different types of micro-organisms because of its short wave length and its high energy, The biggest/largest UV anti-microbic effect is around 250-260 nm (254 nm) wave lengths [2]. This wave length is the most effective way of absorbing of DNA.

By DNA cells absorbing UV radiation energy, this causes death by damaging DNA cells and its structure [16].

When UV is at the most powerful stage it becomes more damaging, and in this situation its impossible to repair the damaged cells/Structures. When the lighting process is being increased or becomes more powerful (bigger voltage, source of light) the number of dieing vegetative cells increase.

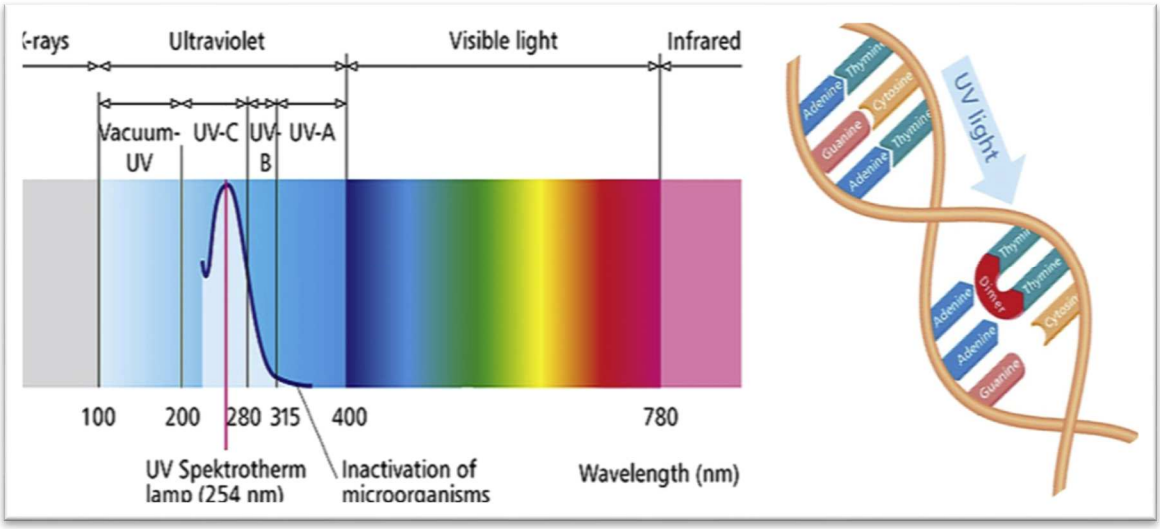


Figure 2.1 Efficiency rates between UVA-UVB-UVC on bacteria death [1].

The UV lamps are very helpful for disinfection and sterilization, nevertheless they are harmful against human health. Because of this, humans should avoid to be in close areas where UV rays are actively used. The negative effects of the UV waves on eyes and the skin was proven scientificly.

UV systems are for disinfection and sterilization of use, there are major benefits compared to other chemicals. These;

* Easy to apply

* Disinfection abbility is high

* The enviroment / or surface does not generate by-products and residual substances

* There is no formation of corrosive substances

* It's economical

UV lamps not only affective in endustrial areas and hospitals[17] but aslo affective in kindergartens, where people in one crowd, cafeterias and in a confined space such as

gymnasiums, as it is transmitted through the air it reduces the number of airborne pathogens which can be used to prevent the spread of disease[18].

CHAPTER 3

MATERIAL AND METHODS

3.1 Ultraviolet Light Source

UV light source used in, light glass electricity with low-pressure mercury with vapor flowing through a tube. Thanks to the flow of UV light produced. These lamps are called as UV lamps. UV lamps operate the same way as fluorescent lamps used for lighting. These lamps like high-energy pulses which emit energy in microorganisms and cells of micro-organisms can cause serious overheating fragmented. When using this method, it is observed that the appropriate micro-organisms and spores reduce organic compounds. By using UVC, we are available to occur an environmental free area from micro-organisms (air and surface disinfection in operating rooms, laboratories and biological safety cabinets)[17, 19, 20].

We used a UVC light that has 254nm wavelenght, 240 mm leght, 15mm diameter, 11 watt power dissipation (Manufacturer: Lightest Lamp Technology, Ltd. Hungary).

Figure 3.1 Ultravıolet Lıght Lamp

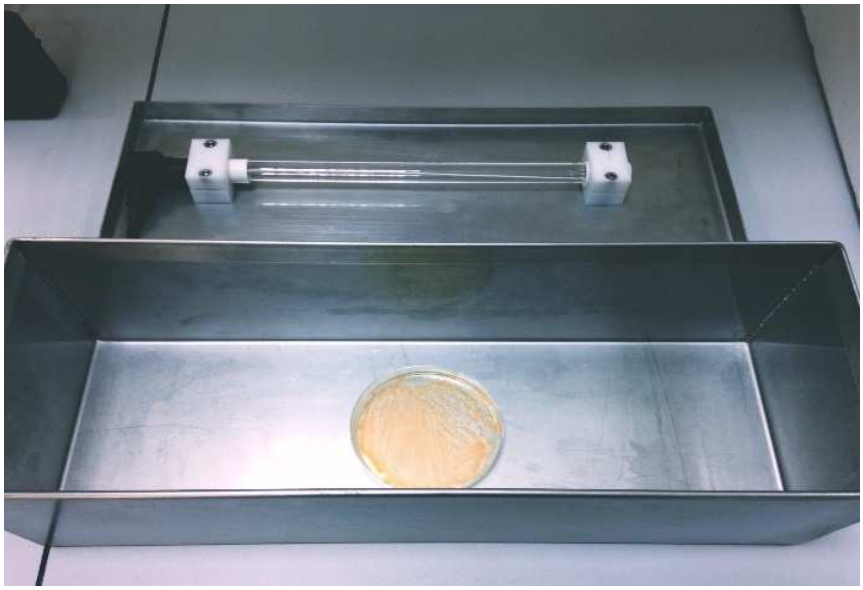


Figure 3.2 Experiment equipment that has UVC lamb

3.2 Experimental Procedure

Figure 3.3 Tryptic Soy Agar and Tryptic Soy Broth

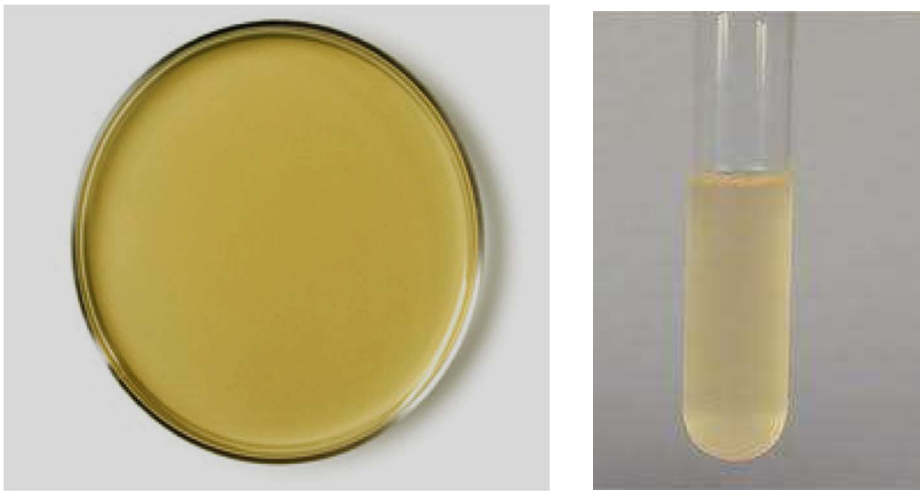


Figure 3.4 Tryptic Soy Agar and Broths Preparation for *Pseudomonas aeruginosa*

Tryptic Soy Agar (TSA) and Tryptic Soy Broth (TSB) are growth medias for culturing of *Pseudomonas aeruginosa.*

4 gram of Tryptic Soy Agar culture medium was homogenized with 100 ml distilled water and autoclaved at 121 °C approximately 20 minute during this procedure. After autoclave became unlock sterilized liquid culture medium embedded in empty cell culture dishes. This process was performed next to bunsen burner in order to protect sterilization. After the liquid in the dishes frosted, the dishes were placed in the refrigerator which was adjusted at +4 °C.

TSB liquid medium was made ready for *Pseudomonas aeruginosa* cultural growth increase and dilution processes. As the agar medium, TSB medium preparation was almost the same which was 3 gram of TSB culture medium homogenized with 100 ml distilled water and autoclaved at 121 °C 20 minute as well. The main difference between the preparation method of TSA and TSB, sterilized liquid form of TSB was not embedded in cell culture dishes. TSB stored in its own glass due to avoid potential contamination risk. After the sterilization procedure of TSB medium, getting cooler and stored under the same conditions as TSA in the refrigerator.

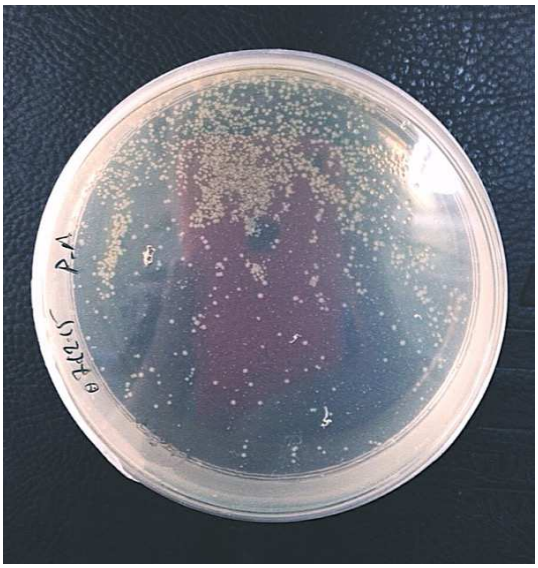


Figure 3.5 Cultured Pseudomonas Aeruginosa Bacteria (Main Stock)

In order to obtain the purest bacteria, *Pseudomonas aeruginosa* that was taken from freezer at -80 °C was waited almost half an hour into the ice until the strain decomposed. Then, *Pseudomonas aeruginosa* bacteria was taken and expanded to cell culture dish that contains TSA with using streak plate technique through the instrument of disposable cell scraper and incubated by incubator for 24 hours at 37°C. This cell culture dish was used as main stock. Every counts involve 10^9 colony in this main stock.

Figure 3.6 Centrifuge Tube Includes Pseudomonas Aeruginosa and Tsb

A colony from main stock was taken and placed into a centrifuge tube that contains 1000 µl TSB by pipette. At every turn, 10 µl was taken from centrifuge tube and diffused to

two separate TSA culture medium and put into the incubator at 37°C to wait for 24 hours. On the following day, one of these two culture mediums was separated as stock, other one was entreated with UV. At the beginning of each experiment, new colony was taken from main stock, dissolved in 1000 µl TSB and new culture mediums were prepared for new transaction.

In the statistical analysis, a confidence level of 95% was used, and differences between groups with $p<0.05$ were considered statistically significant. The data were analysed using the SPSS (v.13.0) statistical package (SPSS Inc., Chicago, IL).

CHAPTER 4

RESULTS AND DISCUSSION

RESULTS

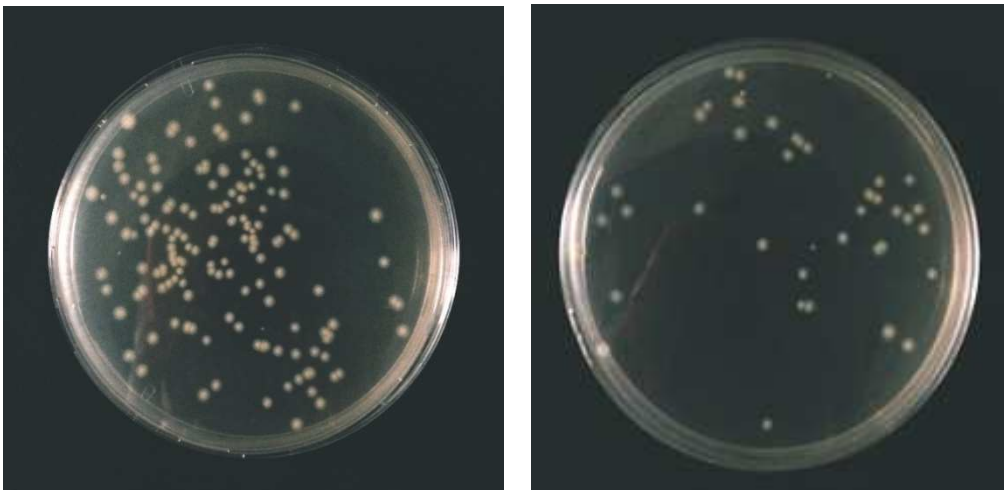
A colony, which has 10^9 bacteria, from main stock was taken and placed into a centrifuge tube that contains 1000 µl TSB. At every turn, 10 µl was taken from centrifuge tube and diffused to two separate TSA culture medium and put into the incubator at 37°C to wait for 24 hours. (now, each colony has 10^8 bacteria)

On the following day, one of these two culture mediums was separated as control, Other one was entreated with 10 minute UV. At the beginning of each experiment, new colony was taken from main stock, dissolved in 1000 µl TSB and new culture mediums were prepared for new transaction. A colony that was received from entreated dish with 10 minute UV and control one, they were stirred in centrifuge tubes with 1000 µl TSB seperately and this dilution procedure was repeated 5 times by taking 10 µl from each ones to obtain the clear number of colonies. (after the 5 times dilution procedure, each colony has 10^3bacteria)

The last mixtures were diffused to a new TSA culture mediums seperately and they were put to the incubator at 37°C to wait for 24 hours. Finally, on the following day, counting process was done.

There is a statistically significant difference between control and ultraviolet experiment results ($p=0.0000056$).

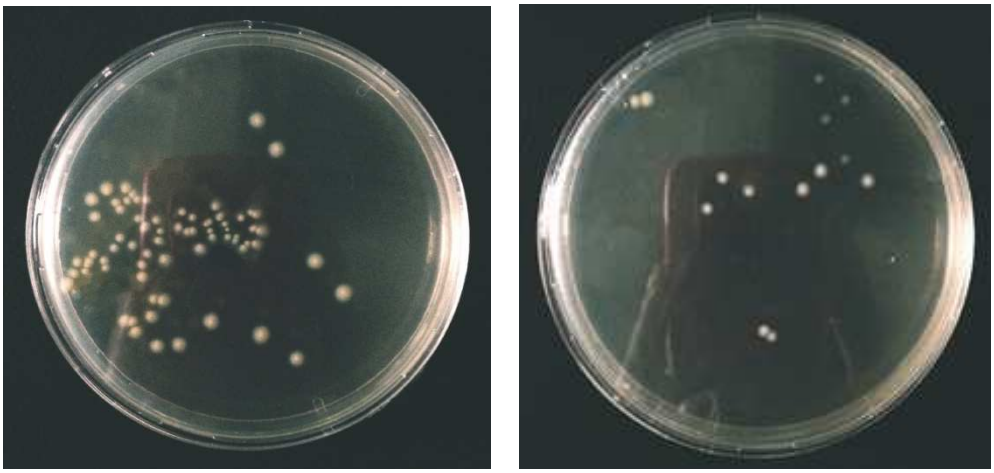
CONTROL 5/1000 **UV 5/1000**



133 X 10^3 Bacteria **36 X 10^3 Bacteria**

Figure 4.1 Experimental Results -1-

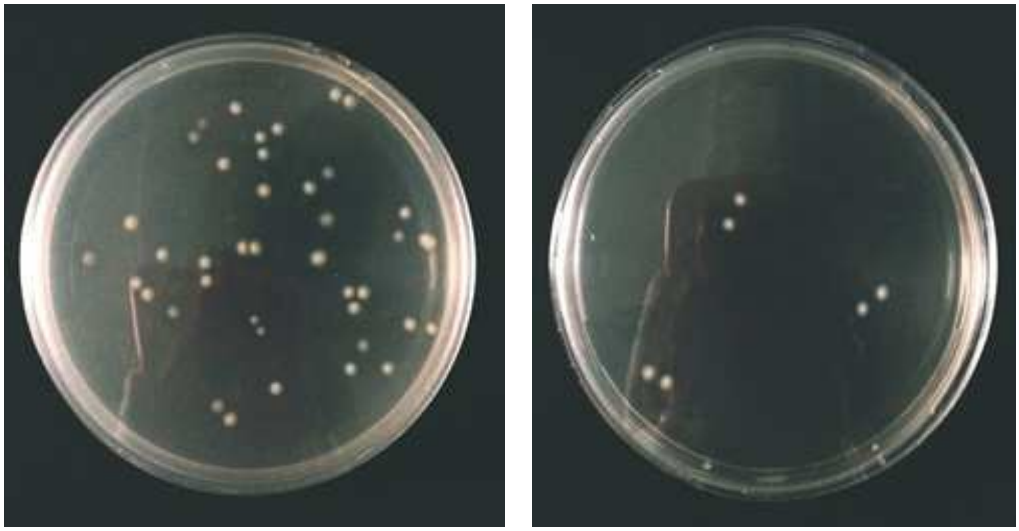
CONTROL 5/1000 **UV 5/1000**



65 X 10^3 Bacteria **14 X 10^3 Bacteria**

Figure 4.2 Experimental Results -2-

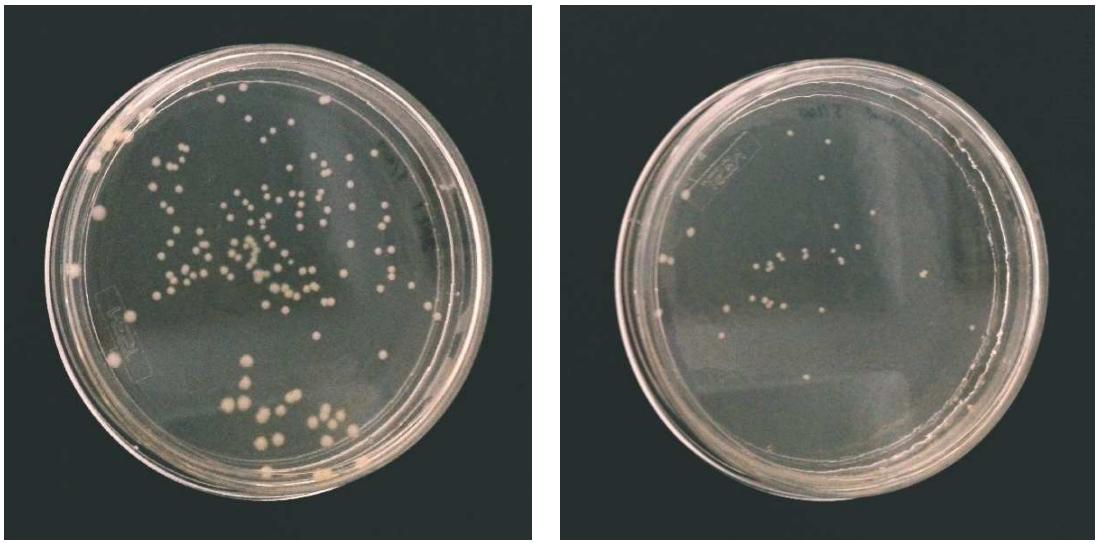
CONTROL 5/1000 **UV 5/1000**



41 X 10^3 Bacteria **6 X 10^3 Bacteria**

Figure 4.3 Experimental Results -3-

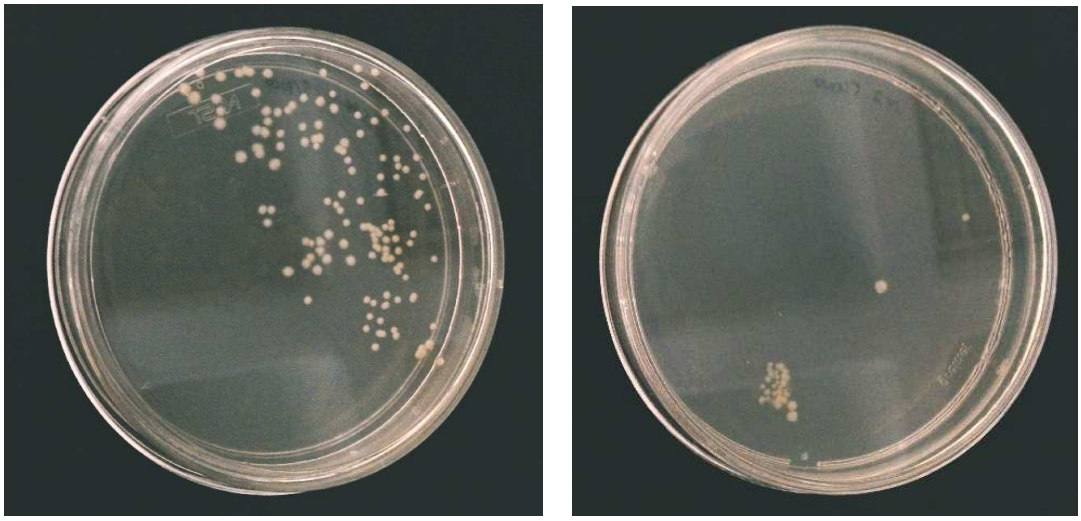
CONTROL 5/1000 **UV 5/1000**



142 X 10^3 Bacteria **34 X 10^3 Bacteria**

Figure 4.4 Experimental Results -4-

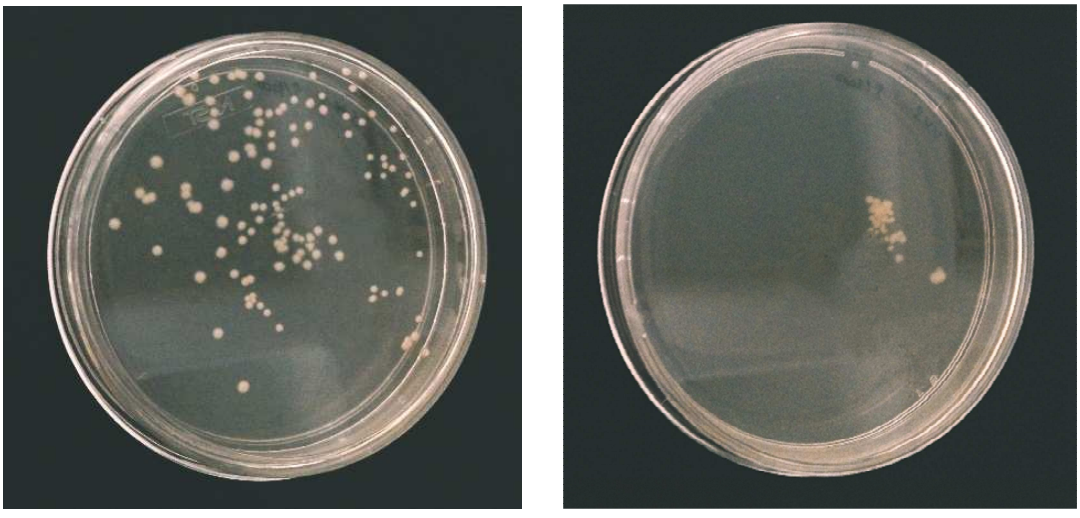
CONTROL 5/1000 **UV 5/1000**



127 X 10^3 Bacteria **33 X 10^3 Bacteria**

Figure 4.5 Experimental Results -5-

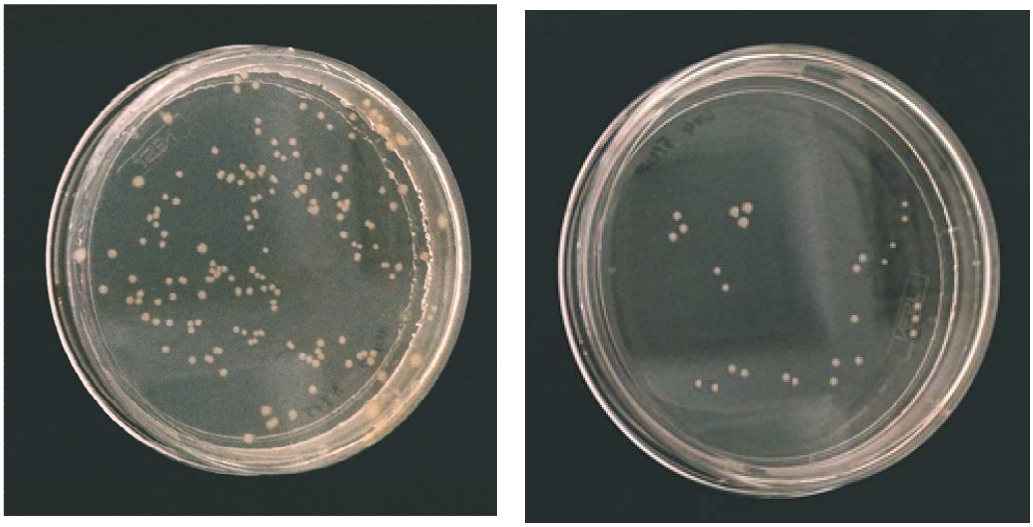
CONTROL 5/1000 **UV 5/1000**



119 X 10^3 Bacteria **22 X 10^3 Bacteria**

Figure 4.6 Experimental Results -6-

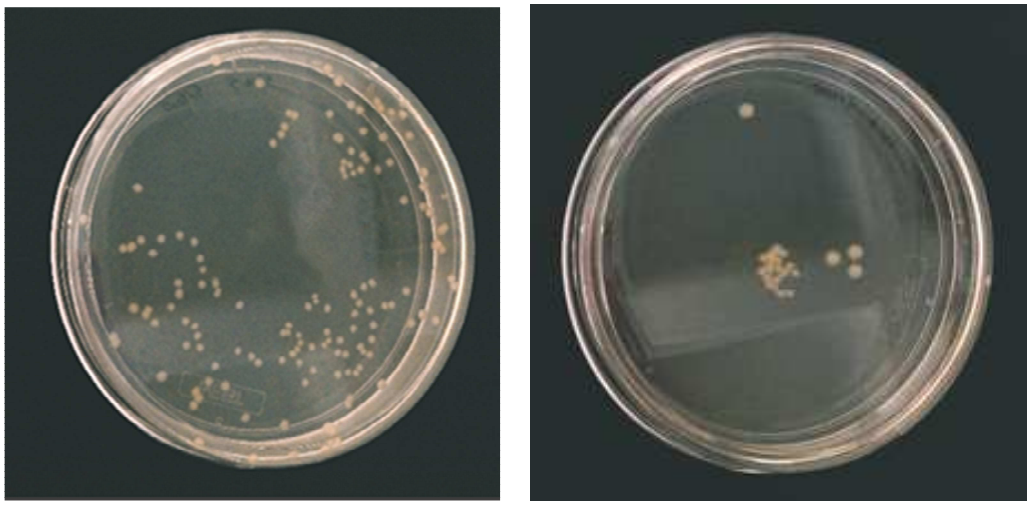
CONTROL 5/1000 **UV 5/1000**



149 X 10^3 Bacteria **27 X 10^3 Bacteria**

Figure 4.7 Experimental Results -7-

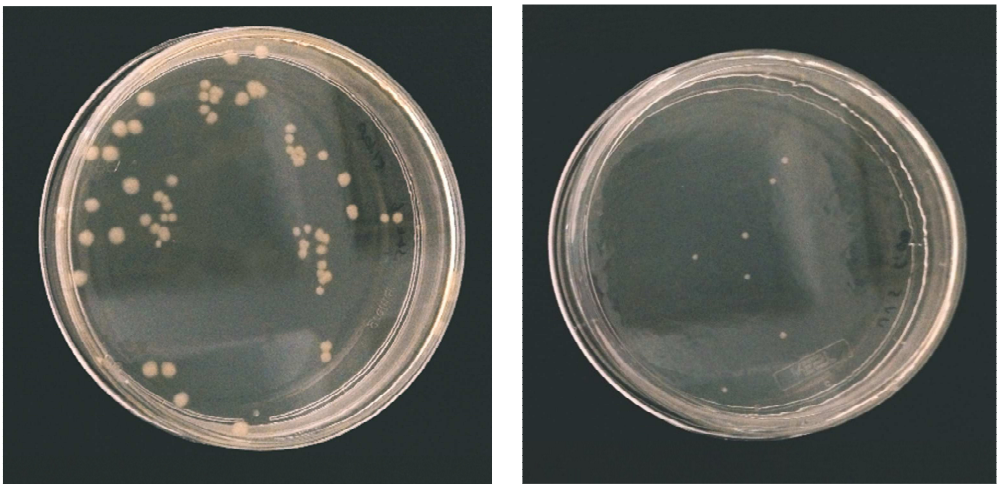
CONTROL 5/1000 **UV 5/1000**



133 X 10^3 Bacteria **21 X 10^3 Bacteria**

Figure 4.8 Experimental Results -8-

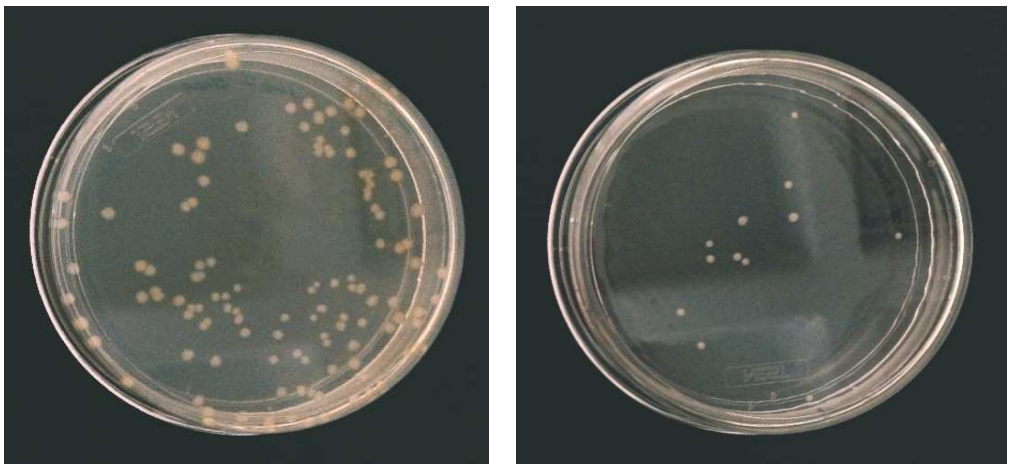
CONTROL 5/1000 **UV 5/1000**



64 X 10^3 Bacteria **7 X 10^3 Bacteria**

Figure 4.9 Experimental Results -9-

CONTROL 5/1000 **UV 5/1000**



114 X 10^3 Bacteria **12 X 10^3 Bacteria**

Figure 4.10 Experimental Results -10-

Experiment No	CONTROL PLATE (X 10^3 Bacteria)	UV IRRADIATED PLATE (X 10^3 Bacteria)
1.	**41**	**6**
2.	**64**	**7**
3.	**65**	**14**
4.	**114**	**12**
5.	**119**	**22**
6.	**127**	**33**
7.	**133**	**21**
8.	**133**	**36**
9.	**142**	**34**
10.	**149**	**27**
Mean ± Std	**108,7 ± 37,8**	**21,2 ± 11,2**

Table 4.1 Experimental Results (Irradiated and Control Group)

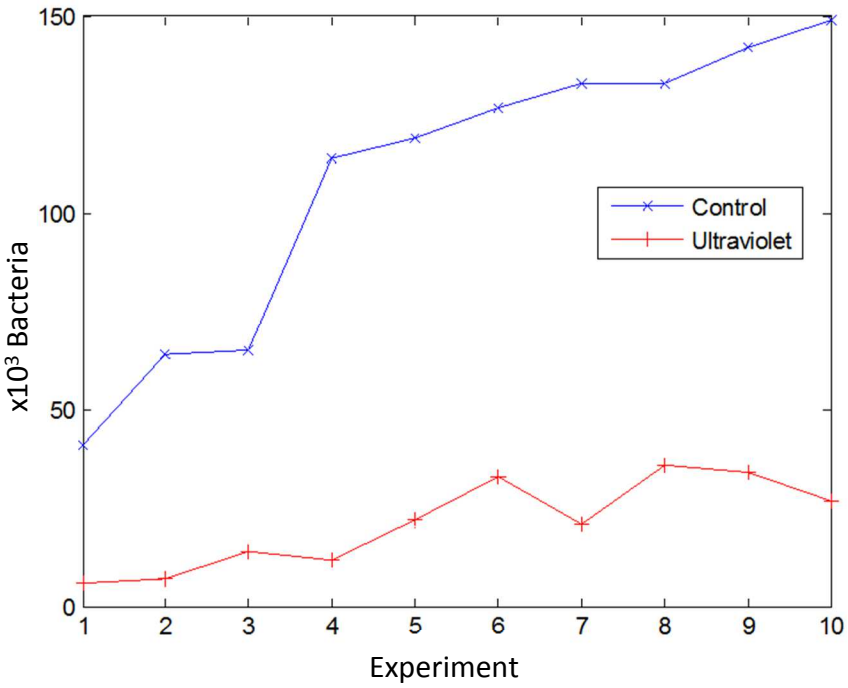


Figure 4.11 Graph, Control Versus UV Irradiated Group Viability

DISCUSSION:

In this thesis work, an experimental setup is prepared to verify and evaluate the effectiveness of ultraviolet light as a disinfection method. 10 pairs of experiments are performed with different amount of bacteria. In each experiment, a pair of identical setup is used except that in each of the pair one plate is exposed to ultraviolet light for ten minutes. Regarding the fact that ultraviolet and control groups have a statistical difference in terms of remaining bacteria, ultraviolet light is found to be effective in disinfection. In our experiments, 4-9 times lower levels of bacteria is obtained.

In the literature, there are many different studies that examine disinfection application of ultraviolet light. Some of the studies interested in drinking water treatment [1, 7, 21]. They examine the effectiveness of ultraviolet light in disinfecting the bactaria, protozoa and algae. Most of them indicate that ultraviolet light can achieve a disinfection ratio better than %99. Some other studies, on the other hand, are related to waste water disinfection [5, 8, 22]. They claim that ultraviolet light disinfection is a sufficient method for waste water treatment before discharge to seas or use in agriculture.

Ultraviolet light is a good tool for medium disinfection. These mediums include surgery rooms [23], hospital rooms [4, 16, 24], public transportation vehicles [25] and so on.

Ultraviolet light, as a disinfection method, is relatively cheaper than most of the other methods. It’s initial cost is low (approximately 40$ in our case), and lamp life time is long (9000 hours). For this long time, there is no cost other than energy dissipated by lamp, which is 11watts. There is a new emerging technology, namely UV-LED, which has lower energy consumption; though it’s initial cost is much more than ours (fluorescent based UV lamp). But it’s efficiency will probably drive the technology to be manufactured with comparable costs in the near future.

Although UV light disinfection is efficient and useful, it has some safety concerns. Especially the wavelength used in this study (254 nm) is harmful for other living organisms including human. Skin damages, even skin cancer, and retinal damage, even blindness are some of the risks of UV light. To avoid these risks, precautions must be taken to prevent human exposition to UV light sources.

To sum up, according to the results that are observed after the experiments the ultraviolet disinfection is a suitable disinfection technique for non-viable biomaterials. The cost of the ultraviolet lamps, ease of operate are other adventages of this technique.

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