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Experimental Analyses of an Atmospheric Pressure Electrical Plasma Decontaminator

Eric Pius Pradeep
University of Tennessee - Knoxville

To the Graduate Council:

I am submitting herewith a thesis written by Eric Pius Pradeep entitled "Experimental Analyses of an Atmospheric Pressure Electrical Plasma Decontaminator." I have examined the final electronic copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Engineering Science.

Igor Alexeff, Major Professor

We have read this thesis and recommend its acceptance:

J. Douglas Birdwell, Mohammed Ferdjallah

Accepted for the Council:
Carolyn R. Hodges

Vice Provost and Dean of the Graduate School

(Original signatures are on file with official student records.)

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Vice Chancellor and
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Experimental Analyses of an Atmospheric Pressure Electrical Plasma Decontaminator

A Thesis

Presented for the

Master of Science

Degree

The University of Tennessee, Knoxville

Eric Pius Pradeep

December 2006

Dedication

I dedicate this work to my parents, Mr. Michael Dhanraj and Mrs. Amaly Dhanraj, who have always believed in me and stood beside me in all decisions I have taken, accepting financial burdens to help further my education; and my mentor Dr. Igor Alexeff, whose continual guidance and motivation has aided me to pursue and achieve this degree. The empathy, love and support of these people have goaded me to accomplish this grandeur feat. Thank you to all of you again.

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On this special occasion I would like to express my appreciation for my friends in Knoxville, TN, especially Shankar Narasimhaswami, Sri Harish Valluru, and Suman Duvvuru who have made my life in the United States much easier and enjoyable.

Lastly, I take this prospect to thank everybody who directly or indirectly was involved in the research and documentation of my Masters thesis.

Abstract

Electrical Gas Plasmas have been widely utilized in the frontiers of sterilization and disinfection of hazardous chemicals and unsafe microorganisms. In this research Atmospheric Pressure Non-thermal Plasma has been employed using various working gases such as air, helium and nitrogen to study its decontamination effects. Focus is given to analyze the constituents of the plasma such as UV light, charged particles, and chemical components produced during the operation of the discharge which influences the incapacitation of the exposed entities, in this specific study, only bacteria and bacteriophages were exposed. Biological tests involved the cultivation of bacteria and bacteriophages on Petri dishes and their corresponding viability analysis after exposure to the plasma discharge, which was performed in an effort to realize the effectiveness of the treatment using the sterilizer. The results from the experiments were very encouraging in terms of debilitation, energy consumption and time requirements offering a comprehensive explanation of the efficiency of the sterilizer and its potential applications in various areas which includes military, medicine, post offices, hand sanitizers, recycling of hazardous wastes and many more. Use of Hydrogen Peroxide as a moistening agent in the discharge instead of water has yielded some astonishing results concerning the death rate of the microorganisms. Most importantly its prospect of being developed into a large scale medical sterilizer are reasonably high due to the fact that it does not need intricate operating regimes and therefore installing and operating the sterilizer will not require medical or engineering proficiency.

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Chapter 1

Introduction

1.1. Necessity and Current Trends in Sterilization

Sterilization is a process, physical or chemical, performed for the elimination of transmissible agents such as bacteria, viruses or for that matter any viable microbial organism from a specific surface, this is quite different from disinfection where in only harmful agents are eliminated. Sterilization in general has no boundaries of application, although it is widely used in Medicine. But in recent days, the world has experienced a lot of danger from bio-warfare, wherein dangerous micro-organisms are cultivated and used for attack on human beings and other life forms, thus making the concept of Sterilization a vital ingredient for the safety of life.

Conventional methods of sterilization include autoclaving, heat ovens, and treating the materials with chemicals, specifically gases such as Ethylene oxide (EtO), in addition Electromagnetic irradiation, exposure to UV, treatment with ozone are also in existence. These methods have a lot of disadvantages and inadequacies including time consumption, process complication, and advanced knowledge of operating procedures, not to mention the harmful effects on human beings, thus giving rise to requirements of faster, efficient, less complicated and all the more imperative - safe alternative means for sterilization. Plasma Sterilization is the technology that the vast majority of the scientific population has turned to – due to its overwhelming advantages. In an effort to study the effects of plasma sterilization and to reason out the ways to better them, this research is

oriented toward the designing and understanding the Atmospheric Pressure Plasma Sterilizer, more importantly, exposing microorganisms to the plasma and analyzing their survival curves. Survival curves are a typical representation of the efficiency of the sterilization techniques as far as microorganisms are concerned because their rates of inactivation are exponential in most cases. In this study the scope is limited to the case of a homogeneous population of bacteria and bacteriophages.

The microorganisms involved in the experiments are Bacterium - *Escherichia coli* (E-coli), and Bacteriophage – *Wild type standard Lambda phage*, which is a virus that has been genetically engineered to infect E-coli specifically. All the microorganisms were cultivated in the University of Tennessee Micro-biology laboratory and the experimental procedures of exposing them to the plasma discharge and development of the plasma sterilizer itself was done at University of Tennessee Microwave and Plasma Laboratory.

Past experimentation with plasma decontamination reveals the influence of various plasma components – for example charged particles, UV photons, chemical components and so on which are involved in the incapacitation of the exposed microorganisms. Therefore, in this research, due importance is given to find out the roles of these "constituents of plasma" and how much impact each has on the debilitation.

It is imperative to understand that the active species in the plasma - ultraviolet (UV) photons and radicals (atoms or assembly of atoms with unpaired electrons) influence the sterilization in a different manner as compared to other methods. One major advantage of the plasma sterilization is its relatively low temperature operation regime ($<50^{\circ}C$), which helps preserve the integrity of polymer-based instruments used in hospitals and other medical research labs that cannot be subjected to autoclaves and

ovens. Furthermore, in medical applications, plasma sterilization is safe both for operator and patient, in contrast to EtO [1].

1.2. Plasma

In general, plasma can be defined as an ionized gas which possesses a neutral collection of ions, electrons and neutral molecules and which is highly responsive to external magnetic and electric fields, they are also termed as "Electrical plasma" or "Gas plasma", although, there is no universally accepted definition for plasma; in fact the name itself instigates a certain amount of ambiguity when it comes to organic and non-organic sciences. Plasmas are attributed as the fourth state of matter besides solid, liquid and gas. The Nobel Laureate, Irving Langmuir, who worked extensively with plasmas in the early 20th century, first used the term "plasma".

Plasmas exist naturally in space; in fact closely 99% of the visible universe is filled with plasma. On earth, plasmas do not exist naturally. Plasmas used in industry and other scientific purposes are artificially generated plasmas called "confined plasmas" and therefore are quite different from space plasmas. These plasmas can be produced by a variety of methods, such as, subjecting a working gas (gases) to an electric field, a magnetic field, and microwave pulsing.

Some interesting properties of plasma that are worth mentioning are stated below: Plasma consists of a collection of free-moving electrons and ions that have lost electrons. Energy is required to strip electrons from atoms to make plasma. This energy can be of any form like thermal energy, light energy (UV or laser), electrical energy. Because they

are highly responsive to electromagnetic fields, Plasma can be steered by electric and magnetic fields which are the sources of confinement of man made plasmas.

1.3. Plasmas in Sterilization - Low Temperature Plasma

Plasmas can broadly be classified into two categories, the high temperature plasmas and the low-temperature plasmas. Plasmas used for sterilization purposes are required to possess low temperatures due to significant advantages as opposed to their high temperature counterparts. This can be achieved by various methods but the most popular method is applying an electric field, whereby ionizing the electrons and ions but maintaining the neutral gas atoms at a considerably low temperature. Typically, low temperature plasmas require a vacuum chamber where all the atmospheric gases have been evacuated to a pressure below 0.1 Torr. The low pressure allows for a long mean free path for the accelerated electrons and ions but since the neutral particles are at ambient temperature there are relatively fewer collisions of the neutral molecules with the electrons and ions which make the plasma remain at lower temperatures. Such electrically induced plasmas are called “Electrical Plasmas” and due to the lower temperatures are preferably referred to as “Cold Plasmas”. The major role players in the plasma energy buildup are the electrons, which due to their low mass, are accelerated to higher energies – sufficient enough to ionize the neutral gas atoms, the values for $\boldsymbol{T_e}$ range from $10^4 – 10^5$ K in cold plasmas. This result in lower energy cost and eliminates the need for external cooling. Although, electrical plasmas or the cold plasmas have certain disadvantages, the major constraint being their degree of ionization, which is

considerably lower than the thermal plasmas (high temperature plasmas). The two most commonly used electrically induced plasmas are the *DC glow discharge*, which is effected by applying a DC voltage to the working gas and the *RF-discharge* which uses a RF electrical field to couple the energy into the discharge [2], [3].

1.4. Atmospheric Pressure Plasmas

As the name itself suggests, atmospheric pressure plasmas generated at atmospheric pressures, their main advantage being that they eliminate the need for the inefficient vacuum systems. It is a well-known fact that low temperature plasma is traditionally generated at reduced pressures but the need for cost-efficient methods for sterilization and decontamination have given rise to Atmospheric pressure plasmas. Methods to generate large volume plasmas at or near atmospheric pressure are numerous, some are, the Dielectric Barrier Discharge (DBD) and the Resistive Barrier Discharge (RBD). The DBD requires frequencies in the kHz range but RBD can be driven by DC or AC power sources, which is the one used in this research. These devices can generate relatively large volumes of non-equilibrium, low temperature plasmas at or near atmospheric pressure which use air or any other gas mixtures as their working medium. The plasmas produced by these devices have gas temperatures generally below 150^{o}C [4], [5]. A basic property of atmospheric pressure plasmas is the high collision rate between species, in particular the collision rate of electrons with neutral species which is greater than 10^{11}/sec making the power absorption not directly dependent on the frequency of the applied field [6].

Atmospheric pressure plasmas can be divided into three major categories, thermal, non-thermal and microwave plasmas. In thermal plasmas all the components of the plasma are in perfect thermal equilibrium. Thermal plasmas include Arc plasmas and their characteristically low neutral gas temperatures and high kinetic temperature of electrons distinguish Plasma torches that have typical temperatures of 10^4 K. Non-thermal plasmas. Non-thermal plasmas include Corona discharge, Barrier discharge and Micro-Hollow Cathode which have temperatures in the order of 10^3 K. Microwave plasmas are capable of producing both Thermal and Non-thermal plasmas depending on their operation setup [4], [5], [6].

1.5. Properties of Barrier Discharges

A widely used source of non-equilibrium plasma at atmospheric pressure is the Barrier discharge (BD) or Dielectric Barrier Discharge (DBD), its working is very simple - an alternating voltage is applied to the electrodes with a dielectric barrier in-between. The voltage induces the dielectric to breakdown when the breakdown voltage of the dielectric is reached and a discharge is initiated. The discharge current is limited due to the presence of the dielectric between the electrodes, thus containing the discharge from transforming into an arc [7]. The barrier discharge can be operated in two operation cycles or modes, namely, the filamentary mode and the diffused glow mode. The generation of the reactive species is controlled by the plasma parameters such as the electric field strength and electron density of the Barrier Discharge. Typical materials used as dielectrics are ceramic, quartz, glass, and Teflon plates, but there are a range of other materials that also can be used [8]. Because of the capacitive coupling between the

electrodes in the DBD, AC voltages or Pulsed DC voltages only can be applied. Another type of Barrier discharge is the Resistive Barrier Discharge (RBD), in here AC or DC voltages can be applied. Although the RBD and the DBD both have specific advantages, the DBD is the most used one [9].

1.5.1. Filamentary Barrier Discharge

Filamentary mode is the normal mode of operation of a Barrier Discharge. When the electric field strength in gap reaches a certain level, the breakdown starts at many points followed by the development of filaments, named micro discharges [9]. The micro discharges are usually distributed uniformly over the surface of the electrode. The breakdown initiating the discharge consists mainly of three phases. They are:

(1) The pre-breakdown phase: A negative space charge of electrons is accumulated in front of the anode depending on the polarity of the applied voltage. The pre-breakdown phase lasts for about half a milli-second. Consecutively, very high local electric field strength is created on the anode and when it reaches a certain critical level, the breakdown occurs.

(2) The propagation phase: It is controlled by an ionization wave, which has high electric field strength. It occurs in the direction to the cathode and therefore pairs of ions and electrons are produced. This phase typically lasts anywhere from 1–2 ns.

(3) The decay phase: It is characterized by the charge accumulation on the dielectric surface compensating the external electric field. It is the period of decay of the light and current pulses of the micro discharges [9], [10].

1.5.2. Diffused Glow Barrier Discharge

The Diffused glow mode of Barrier Discharge is employed in very specific operations. It has some peculiar advantages to the filamentary mode, as in, they are especially suitable for a uniform surface treatment, and also their tolerance with respect to the geometry of the treatment surface is very appreciable. The generation of stable diffused glow Barrier Discharges at atmospheric pressure requires special operating conditions that are mainly determined by the properties of the working gas. There seems to be an occurrence of an effective pre-ionization in Barrier Discharges, as compared to the conditions of the Barrier Discharges in the filamentary mode. The diffuse Barrier Discharge mode is sensitive to impurities and residual ions. Some barrier materials can trap appreciable amounts of charges uniformly on the surface. The required operation conditions can be easily accomplished by using helium, neon and pure nitrogen as working gases. Although, Noble gases can be used as the working gas, and one point worth noting is that they are sources of an intensive short-length excimer radiation. Barrier Discharges produce highly non-equilibrium plasma conditions in a controllable way at atmospheric pressure, and at moderate gas temperature and their capability to treat surfaces at low gas temperature and pressures proves to be a significant advantage over the filamentary mode [8], [10], [11], [12].

1.6. Effects of Plasma on Microorganisms

As mentioned before, the most common method for sterilization used nowadays is ethylene oxide (EtO), especially because polymeric devices are extremely heat-sensitive. This type of treatment with EtO has undergone many environmental regulation changes with reference to chlorofluorocarbons (CFCs), which are used in synchronization with EtO to reduce its flammable properties [13]. Although 100% EtO sterilizers, which no longer use CFCs, have been developed, there are still many questions concerning the carcinogenic properties of the EtO residues adsorbed on the materials during and after treatment [14]. There are also concerns about the safety of technicians because the open and close times of the machines are actually greater than sterilization time [15]. The current trends in sterilizing medical equipments operate with EtO in the gaseous state in spite of its poisonous properties.

These disadvantages are overcome when the proposed electrical gas plasmas are employed. It would be right to summarize the properties of an ideal sterilizer as follows:

1. Low sterilization times and operation regimes.
2. Fewer requirements for technical knowledge for the operation of the machine itself.
3. Lower temperatures and pressures.
4. Capable of operating without using any noxious materials whereby making it safer for the users.
5. Capability to treat a wide range of materials.

Some of the considerable benefits of using Electrical Plasma for sterilization are:

1. Gases involved have no biocidal effect unless they are activated by the electrical power.

2. The reactive species, which induce the killing of the microorganisms, recombine in atmospheric a few milliseconds after the electric field are turned off.
3. The vent time is very short and therefore there is little or no danger for the users.
4. The operating conditions of the plasma can be altered for efficient inactivation with minimal or no damage to the materials subjected to the treatment [16].

1.6.1. Advent of Electrical Plasma Sterilization

Sterilization using plasma started as early as 1968 by Menashi. It employed argon plasma at atmospheric pressure using RF field. Sterilization outcomes were very encouraging. The major notion at this time was that the killing of the microorganisms was caused by the heating effects of the plasma and therefore was also termed *plasma incineration*. At later stages it was proven by various plasma researchers that there are a range of components in the gas plasma that influence the debilitation. Also, different methods to create the plasma were employed; including microwave pulsing, RF pulsing, and laser pulsing and most of these early experiments used inert gases, such as argon or helium. There were also other attempts to use halogens such as chlorine, bromine and iodine within the sterilization chamber to increase the efficacy of the process. But Jacobs and Lin introduced the use of hydrogen peroxide, a sterilant itself. It was realized by experimental techniques that some gases when used in plasma are more efficient than the others. Also, the power density of the plasma was directly proportional to the sterilization efficiency of the system [16], [17], and [18].

1.6.2. Basic Mechanisms of Plasma Sterilization

The basic mechanisms of plasma sterilization as proposed by Moisan et al are as follows:

(1) UV: Destruction by UV irradiation of the genetic material of the microorganism is a process requiring a significant amount of damage to the DNA strands.

(2) Photodesorption: The desorption induced by the photons more specifically from the UV photons break chemical bonds in the microorganism material leading to the formation of volatile compounds inside the microorganism. These volatile bonds then disintegrate, eventually destroying the microorganism.

(3) Etching: This process merely is another method of letting volatile compounds to be formed in the microorganisms; it involves the adsorption of reactive species from the plasma into the microorganism with which they subsequently undergo chemical reactions to form volatile compounds. The reactive species can be atomic and molecular radicals, for example, O and O_3, respectively, and excited molecules in a metastable state. In certain cases, the etching mechanism is enhanced by UV photons [1].

Surprisingly enough, later studies by researchers such as Boucher proved that the inactivation leading to the death of the microorganisms does not change greatly due to the presence or absence of UV photons or UV irradiation. Also, it was suggested that the plasma in fact was emitting radiation from the discharge itself. It was finally concluded that, even though there was a large amounts of UV radiation emitted by the plasma, the

debilitation was not the result of the UV light alone but also is due to the presence of active species such as O, N_2 and so on [18], [20], and [1].

In general, condensation is the basic principle of plasma sterilization. All living microorganisms are hygroscopic; meaning that water vapor in the ambient gaseous phase condenses on their surfaces. A similar condensation principle applies to peroxide vapors like hydrogen peroxide or oxidative gases like ozone, provided the ambient conditions (pressure and temperature) are appropriate. It is necessary to remember the fact that direct exposure to plasma yields shorter sterilization time than exposure to the plasma afterglow, although, the latter being safer and involves less expensive operating costs.

One of the hindering shortcomings of plasma sterilization is its dependence on the actual "thickness" of the microorganisms that are to be inactivated because the UV photons need to reach the DNA and destroy them that implies that any material surrounding the microorganisms increases the time required to achieve sterilization.

1.7. Role of Hydrogen Peroxide in the Plasma Sterilization Process

Hydrogen Peroxide (H_2O_2) is a naturally occurring, colorless, water-like compound that has various practical applications. It is used in commercial applications in various concentrations and depending on the concentration it can be used as a sterilant or a bleaching agent to rocket fuel or explosives. A 3% solution of hydrogen peroxide is used in medical applications as an antiseptic. It is one of the best-known oxidizing agents. In gas plasma technology and other industrial applications the use of H_2O_2 is relatively new. Normally the injection of hydrogen peroxide in plasma brings about the need for a vaporizer or a generator that would vaporize it and mix it with the plasma for

disinfection. This specific form of hydrogen peroxide is termed as vaporized hydrogen peroxide (VHP) or Hydrogen Peroxide Vapor (HPV). It is being used extensively in industries and sterilization techniques; for example, it is used in air purifiers in buildings isolators, medical sterilization and many more. In general, the role of hydrogen peroxide in a plasma sterilization process is as a precursor to the generation of active species. Thus formed HPV in the gas plasma, acts alone or in conjunction with the later formed active species and radicals to bring about the sterilization effect. In this specific type of sterilization mechanism initiation is done to make contact between the materials to be sterilized with the hydrogen peroxide before the generation of plasma itself. In this research the implications of using hydrogen peroxide have been very encouraging, yielding very good kill rates of the bacteria and bacteriophages. The encouraging fact is that the injection method is very simple which is discussed a little later [21], [22], [23], and [24].

Chapter 2

Materials and Methods

2.1. Atmospheric Pressure Plasma Discharge Reactor

The experimental setup of the reactor is particularly simple and easy to operate. The setup consists of top high voltage electrode, grounded electrode, a neon sign transformer and a ceramic slab enclosed in a big wooden box as shown in *Figure 1*. The wooden box has two compartments the upper compartment of the wooden box is where the electrodes, a small air vent – 2cm in diameter and the fan are present. The small hole is for a tube carrying the gas (Ar, He, $N_{2)}$ to be inserted, if air were to be used as the working gas, the opening was closed with a cork. The fan was included in the setup to enhance the circulation of the gas or chemical constituents in the plasma uniformly. The bottom compartment encompasses the 15 KV, 300 mA, 60 Hz neon sign step-up transformer which feeds the power to the discharge, the power to the transformer was the household 110V, 60 Hz AC power supply. The setup has a plastic lid on the top of the wooden box; using which the operator can gain access to the discharge and place the microorganisms that are to be sterilized. The plastic lid has a safety feature, that is, it turns the discharge OFF when the lid is opened.

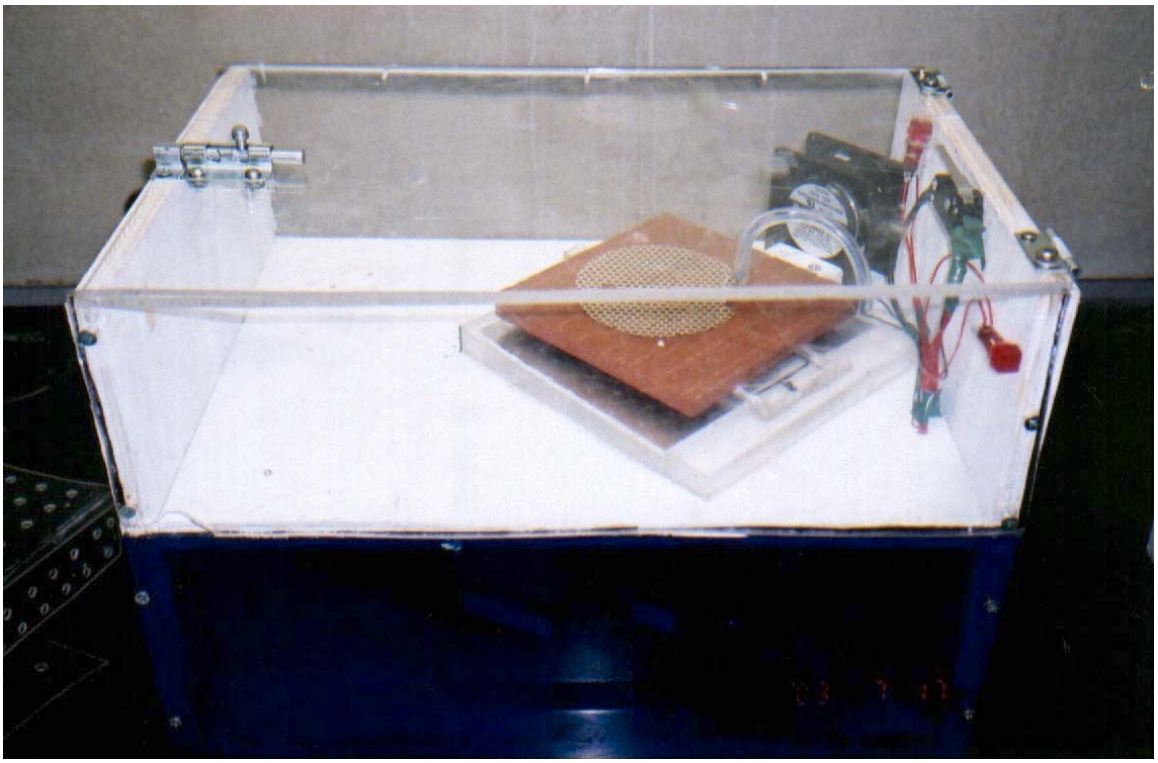

Fig 1: Photograph of the Atmospheric Pressure Plasma Reactor

The high voltage electrode and the ceramic tile (25 x 25 cm) act as a single resistive electrode. The ceramic tile was moistened with water to facilitate the starting of the discharge and to prevent the discharge from operating at high currents whereby avoiding it from arcing. Also, the water induces a cooling effect in the discharge enabling it to run for longer times without heating up and cracking the ceramic slab. In later stages of experimentation, it was realized that using Hydrogen peroxide to moisten the ceramic tiles had much better sterilization effect, which are to be discussed shortly. The electrodes are two 20 cm x 20 cm, square shaped plane electrodes. A schematic of the electrodes and the ceramic are shown in *Figure 2*. The space between them was insulated; the insulator also acts as a support and enables a closed volume of plasma to be formed.

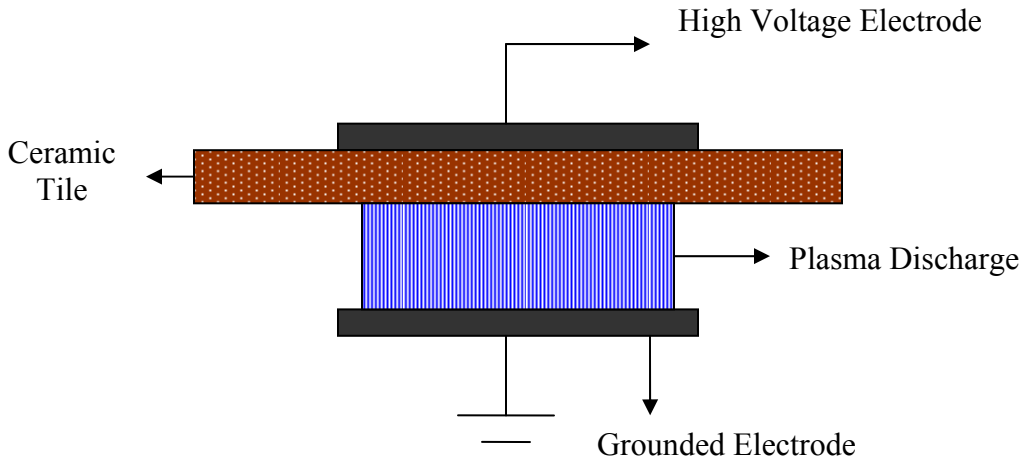

Fig 2: Schematic Representation of the Discharge Setup

As mentioned before the discharge can be operated with air, Ar, He, N_2, or any other gas as the ambient gas. But there are certain points that are to be noted. If air was used as the ambient gas the discharge cannot be large, meaning that the space between the electrodes cannot be made big, no bigger than 10 mm in this specific case, ultimately resulting in small volumes of plasma. On the contrary, it was found that if the noble gases such as Helium or Argon were used as the ambient gas large volumes of plasma could be generated. This finding led to making of a slightly altered setup. The working principle of the discharge remains the same except for some geometrical alterations and the power supply. The geometrical changes were performed to reduce wastage of noble gases and utilize them to the fullest extent generating large cylindrical volumes of plasma.

The supply was a 30 KV, 10 mA DC power supply; this was done to express the versatility of the discharge to take various input power supplies. *Figure 3* shows the working of the discharge with Helium (He) as working gas in the altered setup. In this setup the gap distance can be as high as 15 cm, although, it requires a closed chamber to keep the ambient noble gas in the discharge volume. *Figure 4* shows the discharge operating with air as the working gas. Using plasma diagnostics, it was found that volumes of plasma as high as 1800 cm^3 can be generated with noble gases and 600 cm^3 with air.

Fig 3: Photograph of helium plasma discharge.

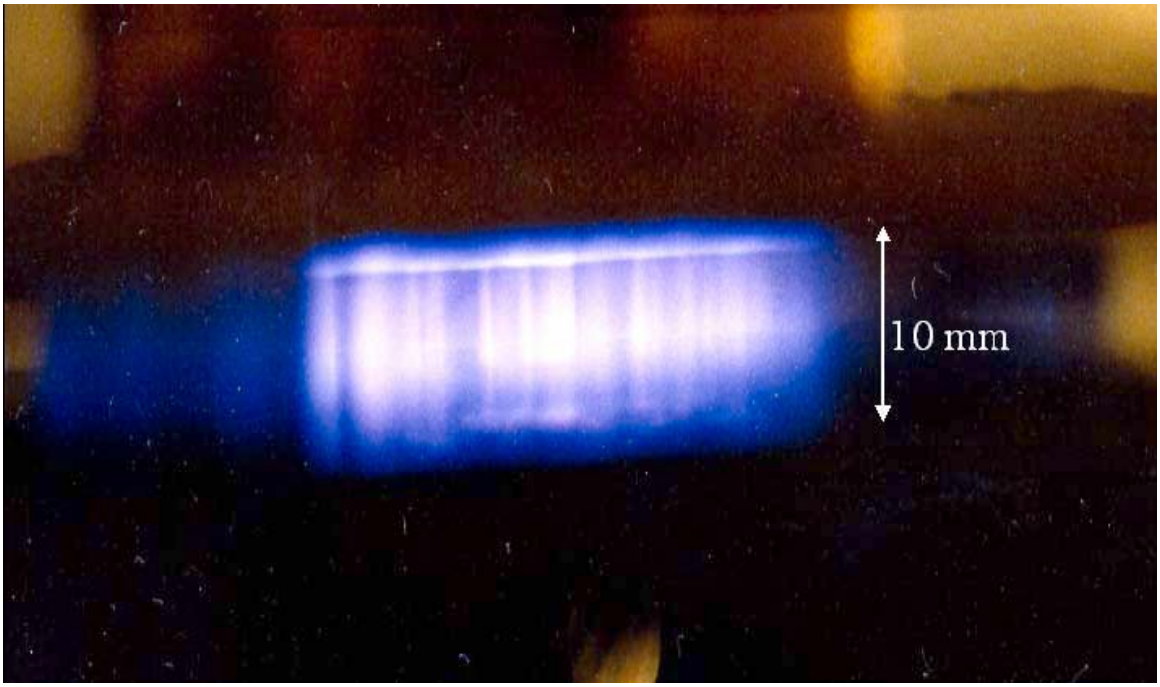

Fig 4: Photograph of air plasma discharge.

2.2. Culture of Microorganisms

2.2.1. Bacteria

Bacteria are a microscopic, mostly unicellular organisms occurring in nature in various forms and shapes and structures. They are prokaryotes meaning that they do not possess a distinct cell structure or nucleus. They lack chlorophyll and reproduce very quickly through fission. They are innumerable types of bacteria, some are useful, some are harmless and some are extremely dangerous and disease causing. Escherichia coli, usually abbreviated to E.coli, discovered by *Theodor Escherich* is one of the main species of bacteria that live in the intestines of many mammals; in fact they are essential for proper digestion. Most forms of E.coli are harmless and because of their ubiquity, have

been studied and documented well. Therefore it proves to be a natural and common choice for most biological studies.

Luria Bertani Broth (LB) – Growth medium

LB broth is a nutritionally rich medium, commonly used for the growth of bacteria. It is also called Lysogeny broth or Luria broth. LB broth recipes for E.coli have been in existence for as long as 35 years or more. The fact is, it still is one of the most common media for cultivating and maintaining various strains of E.coli. General preparation method used in this research is as follows. A one-liter stock solution of the LB medium was prepared by mixing up tryptone (10g), Yeast extract (5g) and Sodium chloride (10g) in one liter of de-ionized water. The mixture was adjusted for a pH value of 7.5 and sterilized by autoclaving at 120^0C for 50 minutes.

LB Pour plates

To the LB medium 17g of agarose - a solidifying agent - was added and gently mixed by rotating ten times in one direction and ten times in the other to obtain LB-agarose pour plate media. The mixture was then autoclaved at 120^0C for 50 minutes after which it was allowed to cool down to room temperature, and then poured into sterilized petri dishes. The LB pour plates thus formed are left to solidify overnight before they are stored at 4^0C for further usage.

Preparation of bacteria (E.coli) for plasma exposure

Master stocks of E.coli are stored at -77^0 C in a culture vial in a 1:1 ratio mixture of 50% glycerol. The next step toward preparation of the bacteria was to thaw the vial

naturally and then suspend it in conical flask containing 100 ml of LB broth along with 100 µl of Kanamycin – an antibiotic. This mixture was then incubated for 24 hours at 38^0C. The antibiotic was added to the preparation procedure to proliferate selective growth of *E.coli* alone on the medium. The particular strain of E.coli used in this research was a genetically engineered strain with Kanamycin resistant gene, meaning that it does not allow any other form of microbial growth on the pour plate medium other than that specific strain of *E.coli*. After the incubation period, 1 ml of this stock solution is added to 100ml of fresh LB medium and incubated for 4 to 6 hours again, before the cells are ready to be harvested. Thus harvested cells were diluted to a 1:10 dilution in six vials, meaning that the dilution factor was 10^{-6} after that 100 µl of the suspension was plated on the LB plates. The dilution factor was derived from trials to be 10^{-6}; it was done to achieve a humanly countable number of bacterial colonies in the plates. The plates were then ready for exposure to the atmospheric plasma discharge. Following the exposure the plates were again incubated at 37^0C for a day or two to detect the viable bacterial count. The colony forming unit (CFU) count was obtained manually.

2.2.2. Bacteriophage

Bacteriophage is a virus that infects bacteria. It is generally termed a phage and they survive on a bacterial host. The phage that was used in this research was Lambda phage (Wild Type standard Lambda Phage). Lambda phage, also called enterobacteria phage is a virus that infects *Escherichia coli*; they are temperate phages, which mean that they are capable of undergoing lysogeny, a type of viral reproduction.

Preparation of XL1 Blue - E.coli based bacterial host for the Lambda phage

XL1 Blue is used as a bacterial host for the Lambda phage. Frozen stocks are processed mostly like bacteria, meaning that they are thawed and suspended in LB medium similarly, except that the LB medium for this specific strain of the E.coli (bacterial host) also has maltose and $MgSO_4$ in it. The mixture is then incubated for 10-12 hours at 37^0C before usage.

Phage buffer

The buffer is made up mixing 150mM Sodium chloride, 40mM of Tris-HCL (pH 7.4) and 10mM $MgSo_4$. Is used to dissolve the agarose and isolate the phage.

LB top agarose

A100 ml stock solution of LB top agarose is a mixture of 1g Bacto-tryptone, 0.5g Bacto-yeast extract, 0.5g sodium chloride and 0.6g agarose. It is added as a second nutrient layer to ease up the process of identifying the plaques.

Plate lysate preparation - Preparation of Phage for plasma exposure

The phage used in the research was Wild type standard Lambda phage. The preparation is discussed in this passage. A fresh culture of an E.coli host strain (XL1Blue) is started by inoculating a single colony into 5ml of LB medium supplemented with 50µl of 20% maltose and 50µl of 1M $MgSo_4$. The mixture is shaken overnight at 37^0 C. A single phage plaque is eluted from an agar plate using a borosilicate Pasteur pipette. The agarose plug is then gently expelled into a 15ml conical tube

containing 1ml of SM buffer and then incubated for an hour at room temperature. About 60-100 µl of elute is mixed with 100 µl of a fresh overnight culture of the bacterial host (XL1Blue). The resulting mixture is incubated at 37^{O}C for 20 minutes. To that mixture 2.5 ml of melted LB top agarose is mixed gently and then poured onto an LB agar plate. After the top agarose has hardened, the plates were inverted and incubated at 37^{0} C for 6 hours or until the plaques have become confluent. Each plate is then filled with 2-3ml of SM buffer before the top agarose (avoiding the bottom agar) is scraped with a spatula and transferred into a high-speed centrifuge tube. Care is taken to break up the agarose using the spatula and then incubated at room temperature for 30 minutes, periodically shaking the tube. The solution containing the scraped top agarose is centrifuged at 8000-10,000xg in a micro-centrifuge for 10 minutes at 4^{0}C. The supernatant is then carefully withdrawn and transferred into a fresh tube along with 0.3% chloroform (v/v). The lysate obtained may be stored at 4^{0} C for up to six months.

Minor procedural changes were done to better study the effects of the plasma discharge on the bacteriophage, such as, the normal procedure proclaims preparing the host and bacteriophage on the pour plates and then exposing them to the discharge. The disadvantage in doing so is that after exposure, one cannot be sure whether the bacteriophage was killed by the discharge or the bacteria died due to the exposure – ultimately causing the death of the phages. So, after a few runs it was decided that the bacteriophages were exposed to the plasma discharge first and then the bacterial host (XL1 blue) was added. This has given a conclusive effect of the apoptosis of the phages.

2.3. Experimental

The objective of this research has been to study and modify the Atmospheric Pressure Plasma Reactor and arrive at an optimal configuration to achieve the best biological decontamination possible. To do this analysis, current performance of the sterilizer has to be realized. Past chemical experimentation at the UT Microwave and Plasma Laboratory by Magesh Thiyagarajan et al reveals the presence of ozone (O_3), Nitrogen dioxide (NO_2) and UV when the discharge is operational. The study was carried out using a gas analyzer to test the amount of ozone and nitrogen dioxide and a photo multiplier to test the impact of UV photons. It resulted in the following observations. When the discharge had just run for about 60 seconds, the ozone production in the discharge was 1.2 ppm and the levels of nitrogen dioxide could not be measured precisely because excessive production of ozone hindered the accuracy of the gas analyzer to detect nitrogen dioxide, but its presence was confirmed.

Ultraviolet (UV), has been a widely accepted and practiced sterilization method for years. Therefore the role of the UV photons had to be analyzed comprehensively. The experiment used a calcite crystal, which essentially is calcium carbonate and a photo multiplier. The calcite crystal is reactive to far UV radiation, meaning that it glows when far UV is present. The near UV was neglected in this issue because it was rendered chemically inactive and has no effect in the sterilization. The results from the study suggest that the UV photons from the discharge are negligible and therefore do not play a major role in the plasma sterilizer's decontamination process.

This being the situation, only possible conclusion is that there have to be other factors influencing the debilitation of the microorganisms. Therefore, this research

approaches this problem in two different ways. One was to find the involvement of other constituents in the plasma, which includes charged particles and OH. The other was to analyze the general efficiency of the sterilizer and to extend its application boundaries.

As mentioned before most of the biological experimentation was conducted using E.coli due to the limitations of the expertise in handling bacteria. One other type of microorganism – much resilient than the bacteria, a phage was also exposed to the discharge, so as to be completely sure of the plasma discharge's abilities in debilitation. The phage is more specifically termed a bacteriophage, meaning that it infects only a specific strain of another microorganism, in this case E.coli, itself.

2.3.1. Active and Passive Exposure Tests

Active exposure is exposure of the bacteria to the plasma while it is working, and passive exposure is exposing the bacteria or the microorganism to be tested to the discharge area after the power to the discharge has been turned OFF and/or by placing the bacterial plates sufficiently away from the electrodes. This experiment was performed to examine the differences with these two exposures because each of them has their own benefits. Some significant advantages of using passive exposure are, it is much easier to be redirected into specific locations using a simple circulation mechanism such as a fan due to the fact that the plasma is not in operation and there is no electrical hazard, also passive exposure is energy efficient because the power is not supplied continuously. This specific test was carried out in the lab as follows. Active plasma exposure was done by placing the Petri dish containing the *Escherichia coli* cells directly below the electrodes at varying time intervals and the indirect or passive plasma exposure was performed by

placing the Petri dish about 10 inches away from the electrodes meaning the plates in this particular case were placed away from the high voltage electrode. The exposure times were in the order of 1, 2, 4, and 8 minutes. A point to be noted is that, in the passive exposure tests the plasma discharge was run for a certain amount of time, more specifically, 1, 2, 4 and 8 minutes without the cells before performing the exact passive exposure tests. This test was designed to study the implications of radiation or high temperature in the decontamination, if any, and it would also give some insight into the sterilizer being a safe means of decontaminating human hands. Some of the applications of passive exposure are hand sanitizers and timed plasma air purifiers that work by alternately turning the plasma ON and OFF.

2.3.2. Nitrogen and Helium Plasma Exposures

This test involves using nitrogen and helium gases as working gases instead of air. The test helps evaluate the characteristics of the sterilizer in the absence of oxygen and its compounds such as ozone. Although it is accepted that there will be production of ozone even when using helium or nitrogen, the quantities were expected to be comparatively less than air. Also, it would give us a clear effect of the volumes of plasma generated and its relation to cell debilitation, if any.

2.3.3. Plasma Diffusion Tests

This test was basically performed to evaluate the perfusion and penetration capabilities of the plasma discharge, so as to characterize its applications in the postal decontamination units, which is gaining a lot of momentum recently. The plan was to

cover the pour plate containing the bacteria with the normal glazed business envelopes and then expose them to the plasma. Since the plasma is non-thermal there is no danger of burning the paper.

2.3.4. Test for Charged Species

The presence of charged species in gaseous plasma is inevitable, but their role in decontamination is not yet clear. There have been various studies involving their dynamics in the plasma, their effects on the plasma confinement and application. In this research, the goal was to test their effect in biological sterilization. A simple experiment was formulated using two cylindrical metal meshes with a diameter of about 6cm which were then introduced into the discharge to act as electrodes. They were placed 1 cm apart with a thin piece of cardboard in-between, for insulation. A 400 V DC was applied between them making them act like an electrostatic filter. The electrostatic filter was placed far away from the plasma discharge to avoid any electrical hazard inside the chamber. It was expected that the electrostatic filter would suck up the charged particles in the discharge chamber, originating from the plasma, and so if the pour plates – i.e. if the bacteria are placed directly under the electrostatic filter; their (charged species) function in the killing of bacteria could be discovered.

2.3.5. Tests with Hydrogen Peroxide

This has proven to be one of the most important findings of this research. The idea of using hydrogen peroxide to moisten the ceramic seems to be a little basic but their implications are astonishing. The plasma discharge is normally operated with water as the moistening agent on the ceramic, but in an effort to analyze the effectiveness of

Hydrogen Peroxide, the ceramic slab was moistened with it. Other plasma researches may have used hydrogen peroxide in conjunction with gas plasmas but they have a complicated injection mechanism for H_2O_2. In this research H_2O_2 is just soaked on the ceramic and the discharge is turned ON, when the ceramic heats up due to the current in the discharge it evaporates the H_2O_2 and mixes the Hydrogen peroxide vapor (HPV) with the discharge. The presence of oxygen and its compounds and their impact on the sterilization have been seriously considered before, but to further analyze the efficacy of having additional oxygen compounds was the major motivation for using H_2O_2 in the experiment. As discussed before, hydrogen peroxide is one of the best oxidizers known to man and it has medicinal applications depending on their concentrations. In this research we used aqueous H_2O_2 in two concentrations, 3% and 30%. This was done to test the possibility of the discharge being used in different applications and also to imply the relationship between the concentrations and the debilitation.

2.3.6. Tests with Bacteriophage

As mentioned before, Bacteriophages are viruses that infect/survive on a bacterial host. The bacteriophage used in this experiment is the *Wild Type Standard Lambda Phage,* which survives on the bacterial host E.coli. This experiment is basically focused at verifying the efficiency of the plasma sterilizer by using it to sterilize the tougher – virus. The phage is grown on LB (*Luria Bertani*) plates and is exposed to the plasma discharge as in the case of bacteria. Here also both water and Hydrogen Peroxide are used as moistening agents to test their effects. The general method of growing phages is to grow the bacteria first and then paste the phage over it before exposure, the disadvantage

in doing so is that it cannot be understood which one (bacteria or the bacteriophage) is killed first, because if the bacteria are killed by the plasma, naturally the phage will also succumb due to lack of a host. To avoid this confusion, the bacteriophage pour plates were treated without the host and then later on the bacterial host is pasted on top of it, this way only the phage is exposed to the plasma discharge and not the bacterial host.

Chapter 3

Results

The results of the experiments discussed in the previous chapter are represented in the form of graphs and tables here. Most of the graphs presented are the survival curves for the microorganisms because these are the widely accepted standards of publishing microbial test results. The tables are presented for the ease of comprehending the numbers. The concentrations of the bacterial solutions and other growth influencing factors such as incubation time, temperature were adjusted so that a humanly countable number of bacteria were present on the pour plates before and after the exposure, approximately 400 ($\pm$ 15) pre-exposure and a tolerance of 0 to $\pm$ 3 post exposure in all cases. It is imperative to keep in mind that the numbers of bacteria were reduced only to ease the counting process and it has no relation to the efficiency of the plasma sterilization, in fact in many cases high concentrations of bacteria were used simultaneously for comparison and still the sterilization was very effective.

The survival curves are but a general representation of the number of bacteria that survive after exposure to the sterilization method, here, the plasma decontaminator with respect to time. All tests were performed in triplicates and the graphs are their average values. Values were averaged due to their minimal deviation. The curve is derived from the simple plate count tests, where the number of viable bacteria called Colony Forming Units and the number of viable viruses called Plaque Forming Units are counted from the pour plates post exposure and incubation.

3.1 Active Exposure Results

The results from active exposure of the bacteria to the plasma discharge show that there is a gradual killing of the bacteria with time. In general, the more the exposure time the more effective the killing, although, complete debilitation was achieved only at or more than 600 seconds. The moistening agent used in this experiment was water. The graph for this experiment is presented in the next page. The active exposure, which was intended as a primary test for the debilitation efficiency of the discharge, was a success, because bacterial apoptosis was observed when the microorganism was exposed to the plasma. It is comprehensible from the *fig 5* that acceptable sterilization was achieved in approximately four minutes. *Table 1* shows the values of the experiments.

Table 1: Viable *E.coli* count after active exposure. Active exposure is placing the bacteria directly under the atmospheric pressure air plasma.

Time of Exposure to the plasma in seconds	Colonies/Colony forming Units – CFU	Survival percentage (%)
0	399	100
60	260	65.16
75	218	54.64
120	86	21.55
240	25	6.02
480	37	9.27
600	9	2.26

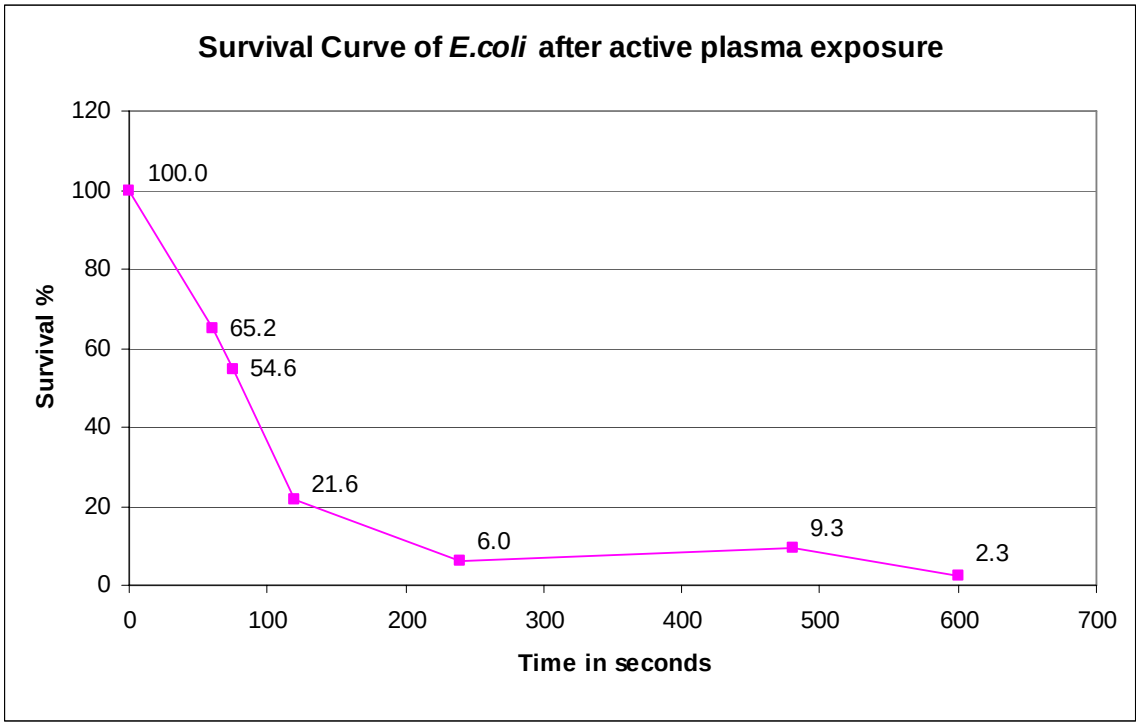

Fig 5: Survival curve graph of *E.coli* after active exposure.

It was understood that further adjustments had to be made to reduce the time required for sterilization and thus giving rise to conduct passive exposures, which are discussed in the next topic.

3.2 Passive Exposure Results

Passive exposures were carried out by placing the Petri dishes away from the plasma and turning OFF the discharge, although, the discharge is run for a specific amount of time, 1, 2, 4, and 8 minutes prior to exposing the bacteria defined as Pre-exposure discharge run time [PERT] to generate whatever it is that causes the killing. The results obtained were quite astounding and are represented in *Tables 2-5* and *Figures 6-9*.

Table 2: Viable *E.coli* count after passive exposure [PERT -1 min]. PERT – Pre Exposure discharge Run Time is to generate passive species.

Time of Exposure to the passive plasma in minutes	Colonies/Colony forming Units – CFU	Survival percentage (%)
0	407	100
1	180	44.23
2	39	9.58
4	85	20.88
8	0	0.00

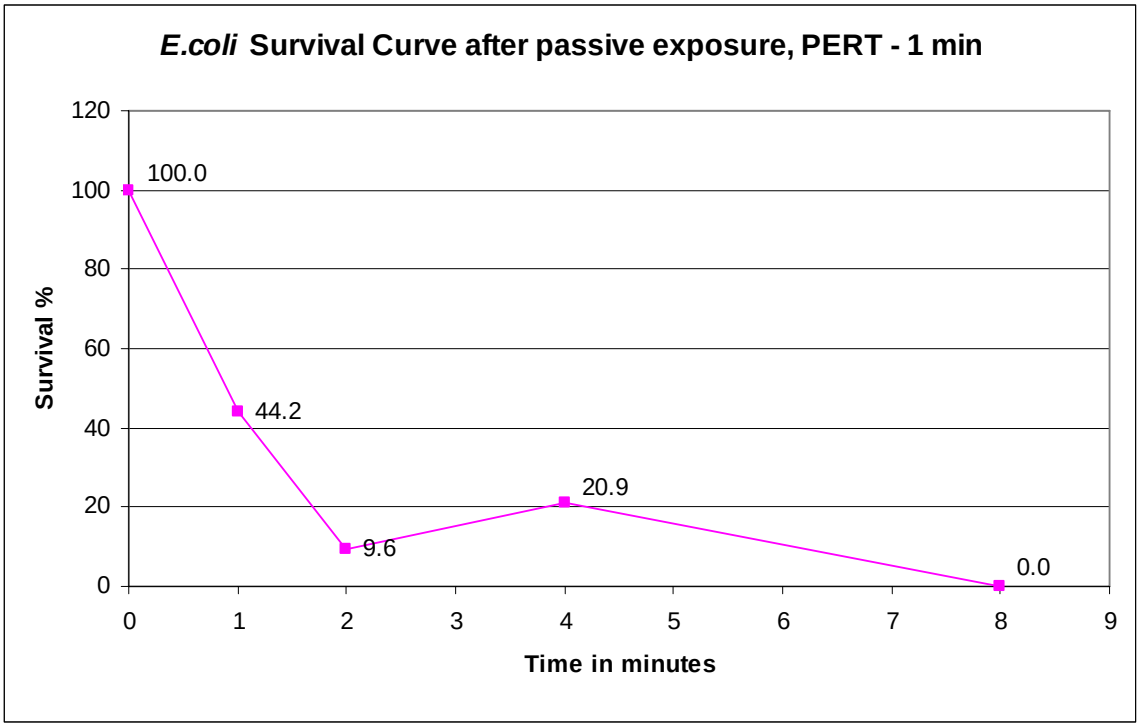

Fig 6: Survival curve graph of *E.coli* after passive exposure [PERT-1min]. PERT - Pre-Exposure discharge Run Time.

Table 3: Viable *E.coli* count after passive exposure [PERT - 2min].

Note: Change in PERT

Time of Exposure to the passive plasma in minutes	Colonies/Colony forming Units – CFU	Survival percentage (%)
0	412	100
1	49	11.89
2	8	1.94
4	0	0.00
8	0	0.00

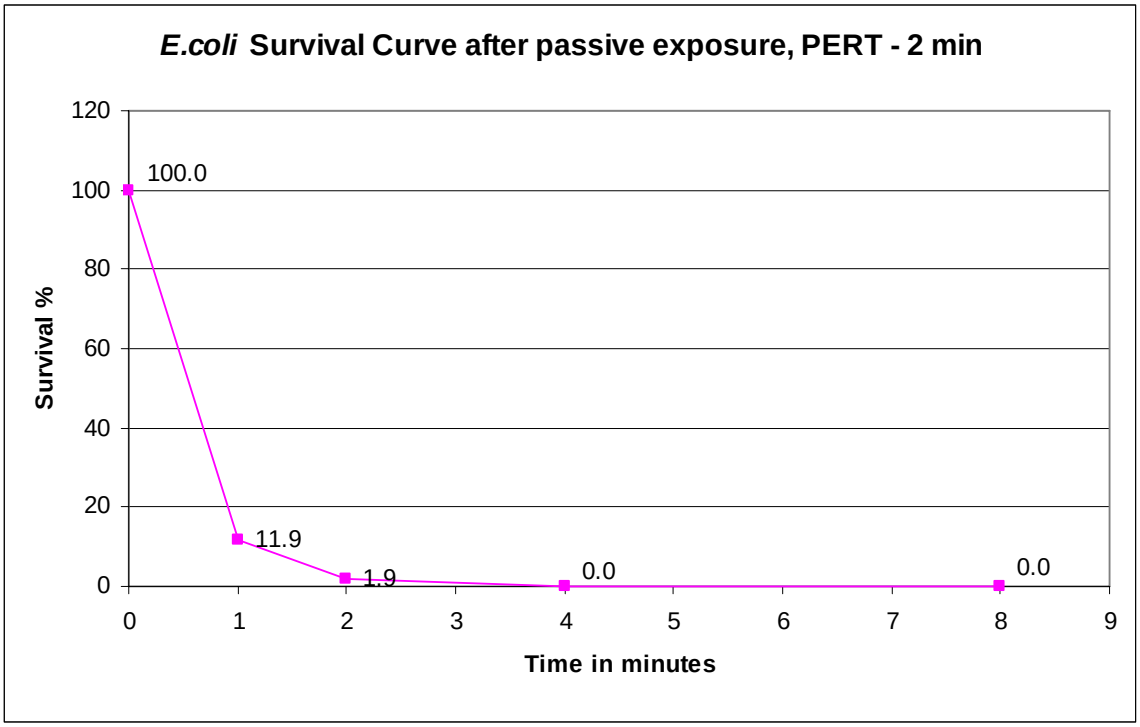

Fig 7: Survival curve graph of *E.coli* after passive exposure [PERT-2min].

Table 4: Viable *E.coli* count after passive exposure [PERT - 4min]. Changes in PERT are made to analyze passive species generation with respect to time.

Time of Exposure to the passive plasma in minutes	Colonies/Colony forming Units – CFU	Survival percentage (%)
0	410	100
1	83	20.24
2	0	0.00
4	0	0.00
8	0	0.00

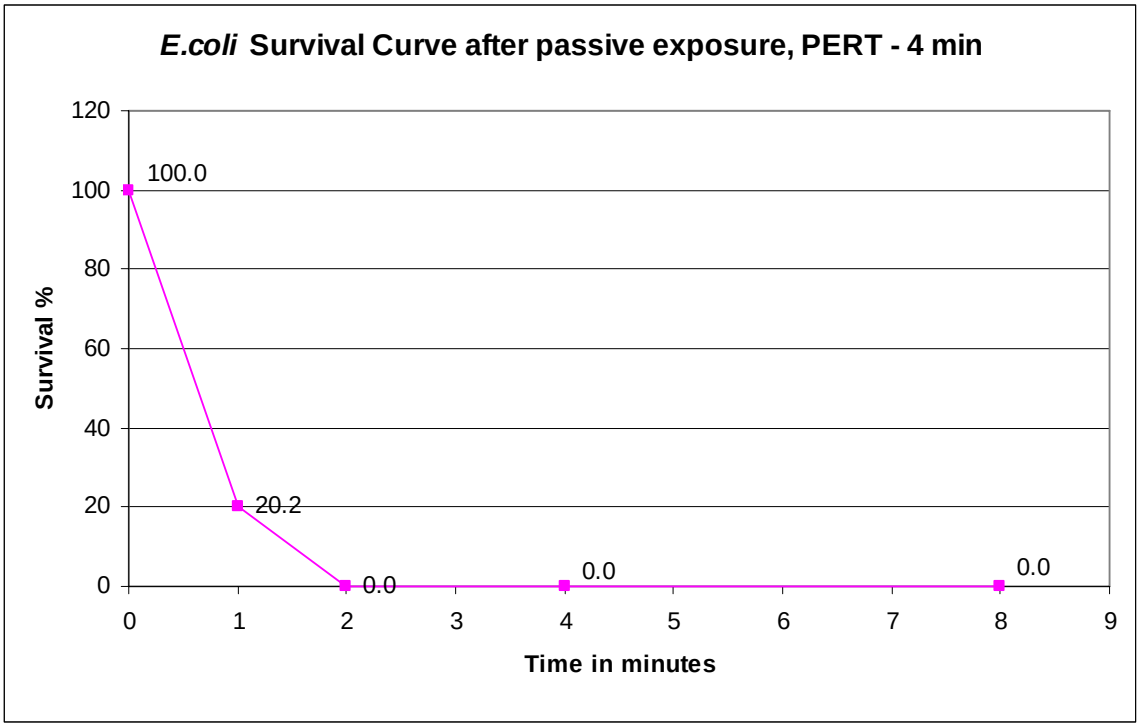

Fig 8: Survival curve graph of *E.coli* after passive exposure [PERT-4min].

Table 5: Viable *E.coli* count after passive exposure [PERT - 8min].

Time of Exposure to the passive plasma in minutes	Colonies/Colony forming Units – CFU	Survival percentage (%)
0	403	100
1	37	9.18
2	0	0.00
4	0	0.00
8	0	0.00

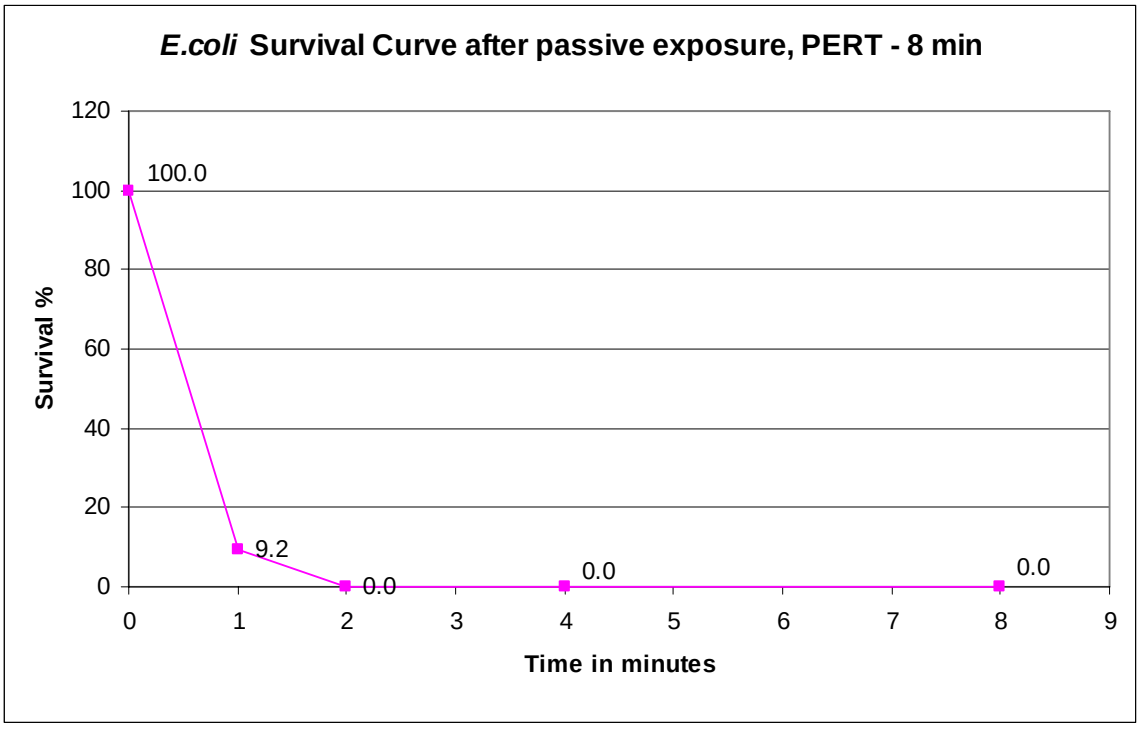

Fig 9: Survival curve graph of *E.coli* after passive exposure [PERT-8min].

Note: Sterilization is getting better.

From the passive exposure tests, it is obvious that using passive exposure is more effective than active exposure, also it is a well known fact that passive exposure requires less power as compared to its counterpart and the major advantage as mentioned before is the technical ease of circulating passive plasma, since it has no electrical hazards. In this experiment we used a fan placed inside discharge. The comparison chart shown in *Figure10* clearly emphasizes the fact that more the pre-exposure run time [PERT], the more effective the sterilization, but it can be seen that effectual sterilizations (below 3% survival rate) are achieved at 2 minutes PERT and 2 minutes passive exposure which are much less as compared to total active exposure times.

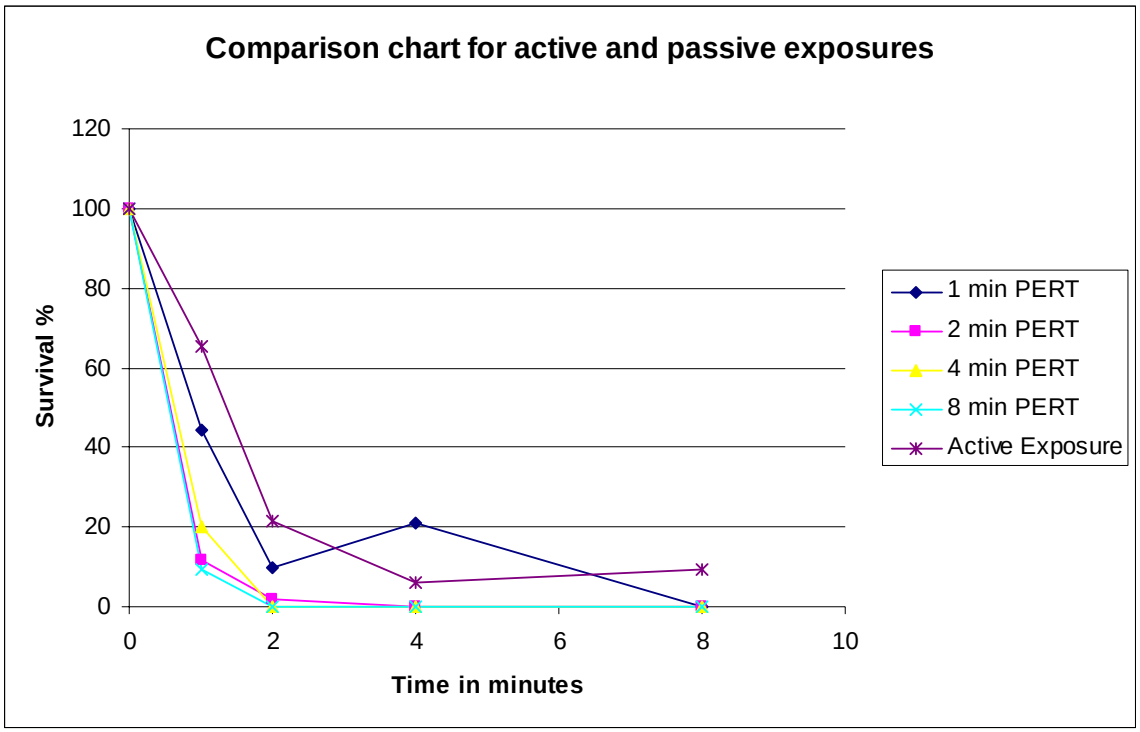

Fig 10: Comparison chart for *E.coli* passive and active exposures. Complete sterilization obtained at 2 minutes PERT and 2 minutes passive exposure.

3.3 Nitrogen and Helium Plasma Exposure Results

This specific experiment was performed to find out whether or not oxygen compounds such as ozone are the only constituents of the plasma involved in debilitation. The principle is based on the fact that helium and pure nitrogen do not produce as many oxygen compounds as air plasma. The results are tabulated in *Tables 6 and 7*, nitrogen first and then followed by helium.

The values depicted in *Table 6* show that there is gradual debilitation with exposure to the nitrogen plasma corroborating the claim that oxygen compounds are not the sole constituents of sterilization in the plasma generated by the Atmospheric Pressure Plasma Reactor.

The graphical representation of the test results is presented in *Figures 11 and 12*.

Table 6: Viable *E.coli* count after exposure to nitrogen plasma. Proves the fact that sterilization occurs when nitrogen is used.

Time of Exposure to the plasma in minutes	Colonies/Colony forming Units – CFU	Survival percentage (%)
0	396	100
1	117	29.55
2	55	13.89
4	6	1.52
8	3	0.76

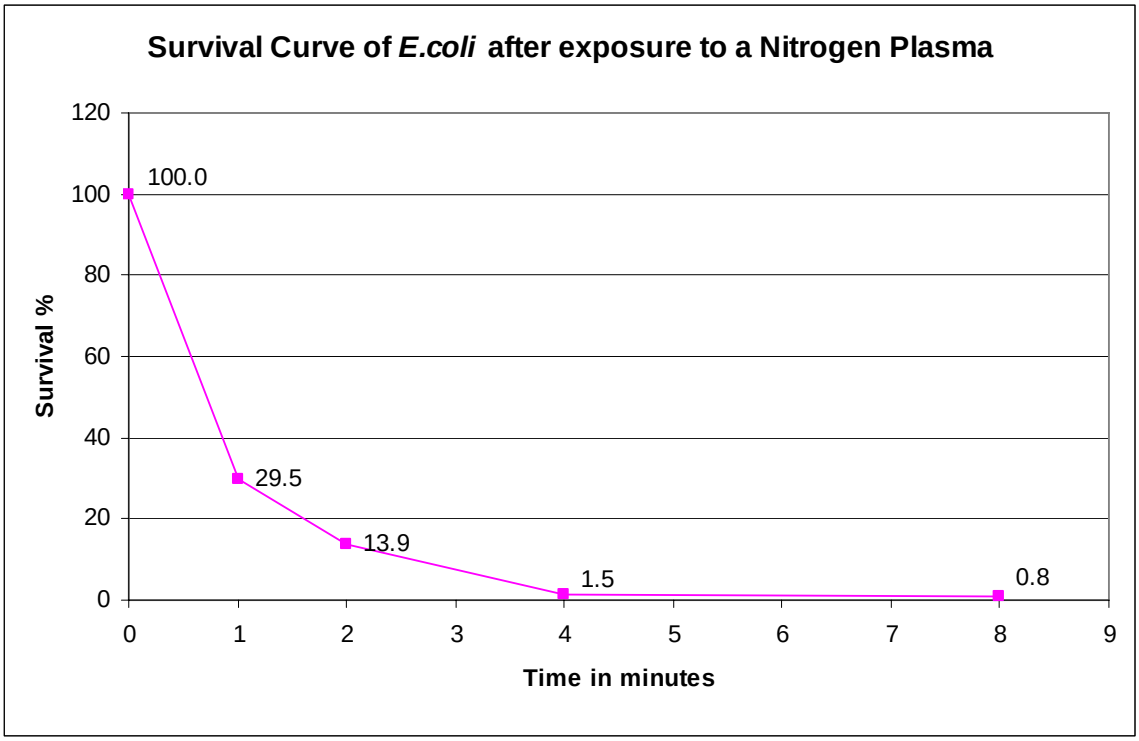

Fig 11: Survival curve graph of *E.coli* after nitrogen plasma exposure. Acceptable values of Sterilization (less than 5% survival rate) were observed at 4 minutes.

Table 7: Viable *E.coli* count after exposure to helium plasma.

Time of Exposure to the plasma in minutes	Colonies/Colony forming Units – CFU	Survival percentage (%)
0	399	100
1	31	7.77
2	44	11.03
4	0	0.00
8	0	0.00

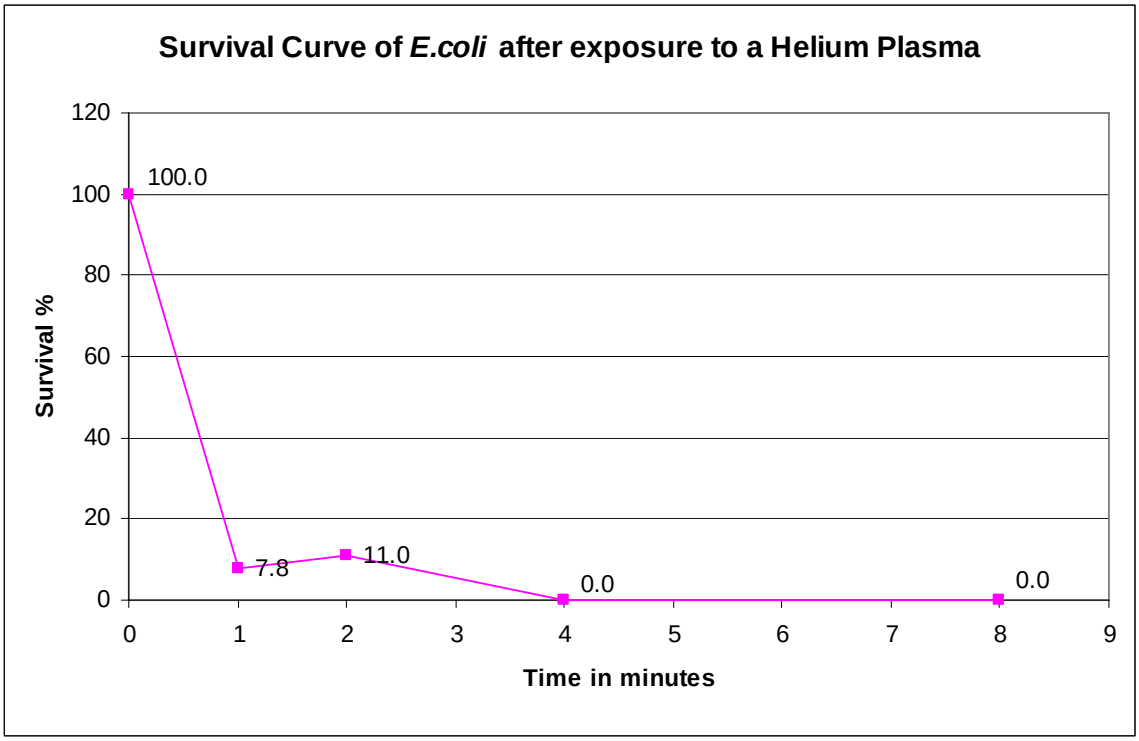

Fig 12: Survival curve graph of *E.coli* after helium plasma exposure. Complete sterilization (0% survival rate) was observed at 4 minutes.

The exposure results to the helium plasma show that there is very fast sterilization. Since the values of ozone have to be less in the helium plasma, it clearly proves that oxygen compounds are not the sole constituents of the sterilization, which is a very encouraging result for this plasma decontaminator. The reason for these astonishing results could be because of the fact that helium is capable of generating large volumes of plasma and therefore more charged species.

A comparison chart of the exposure results of Nitrogen and Helium plasma is presented in *Figure 13*.

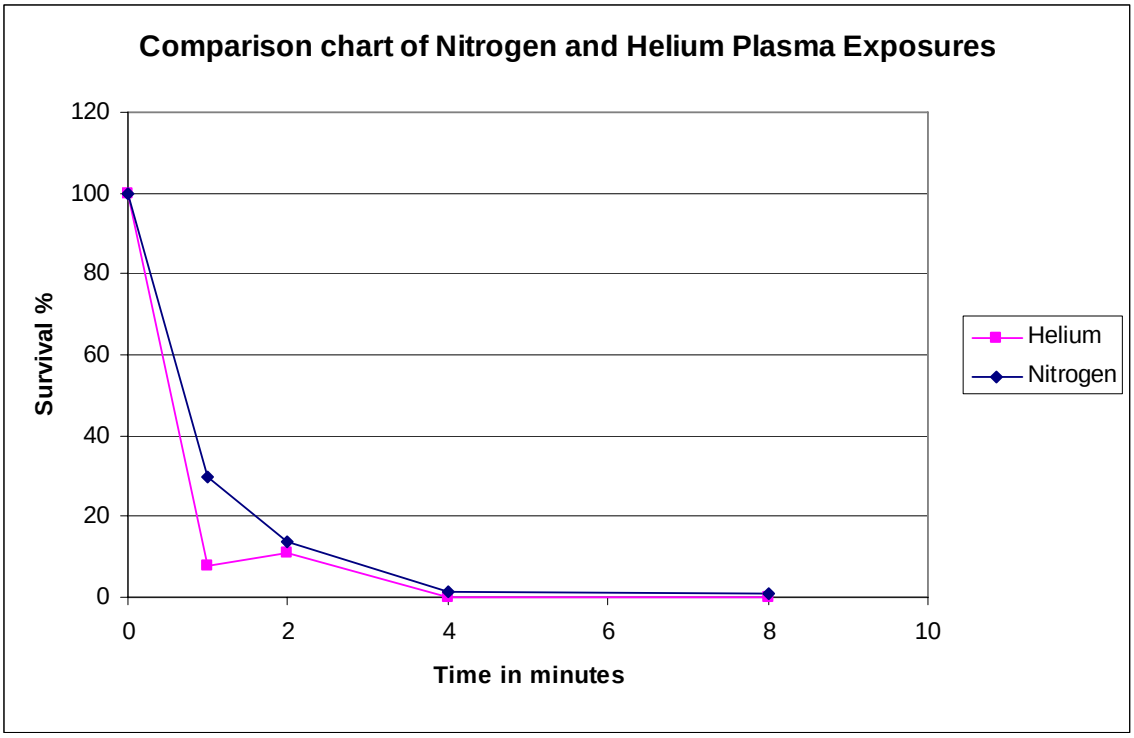

Fig 13: Comparison chart for Nitrogen and Helium plasma exposures.

Note: Helium plasma debilitation clearly better than its Nitrogen counterpart.

From the chart above it is evident that helium plasma has better sterilization qualities than nitrogen. Effectual sterilization (below 5% survival) was obtained at close to 5 minutes in nitrogen and 1 minute in helium, this might reflect on the relation between sterilization and the volumes of plasma generated. Helium when used as working gas produces large volumes of plasma is a known fact, whereas nitrogen's is comparable to air, which might be due to the fact that air is for the most part nitrogen. In any case, the experiments with helium and nitrogen plasma have conclusively proven that there are other factors influencing the debilitation barring oxygen compounds, which is exactly what was expected.

3.4 Plasma Diffusion Test Results

This test was conducted to realize the permeability and diffusion characteristics of the plasma; in this experiment air was used as the working gas and water as the moistening agent. The bacteria were covered all around with the glazed business envelopes and before being exposed to the plasma. The results are tabulated in *Table 8*. The effectiveness of the plasma to penetrate the envelopes is clearly manifested in the results obtained from the experiment. This demonstrates the possibility of using this plasma discharge in postal decontamination. Since the temperature does not rise very much above room temperature in the plasma, there is very little possibility of the paper getting burnt and much less for fire hazards. Although the times needed for sterilization are high, with minor modifications it certainly can be reduced considerably. The survival curve is presented in *Figure 14*.

Table 8: Plasma diffusion test. It shows the number of viable E.coli inside glazed paper envelopes after being exposed to Atmospheric Pressure Air plasma.

Time of Exposure to the plasma in minutes	Colonies/Colony forming Units – CFU	Survival percentage (%)
0	400	100
1	101	25.25
2	47	11.75
4	36	9
8	23	5.75

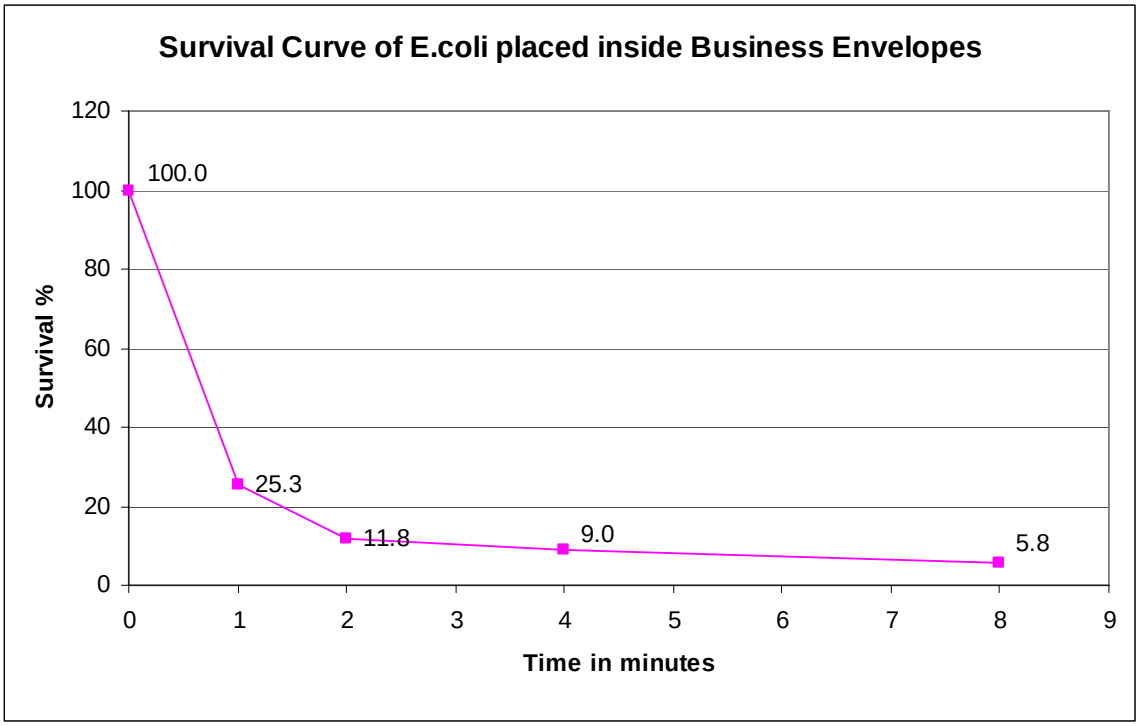

Fig 14: Survival curve graph of *E.coli* placed in envelopes [Diffusion Test].

3.5 Electrostatic Filter – Test for presence of charged species – Results

This test was performed to detect the presence of charged species in the plasma and their role in decontaminating the bacterial plates. The test results are presented in the form of tables and graphs in the following pages with the electrostatic filter OFF in the first case and followed by the electrostatic filter in the ON state. The results show that sterilization occurs even in the presence of the filter, but is the sterilization times are increased considerably as compared to when the filter is OFF, meaning that the charged species/particles interact with the microorganisms in some way or the other. Results are presented in *Tables 9 and 10* and *Figures 15-17*.

Table 9: Viable *E.coli* count with electrostatic filter OFF. It shows the number of viable E.coli after exposure to the plasma in the absence the electrostatic filter.

Time of Exposure to the plasma in minutes	Colonies/Colony forming Units – CFU	Survival percentage (%)
0	405	100
1	380	93.83
2	48	11.85
4	6	1.48
8	0	0.00

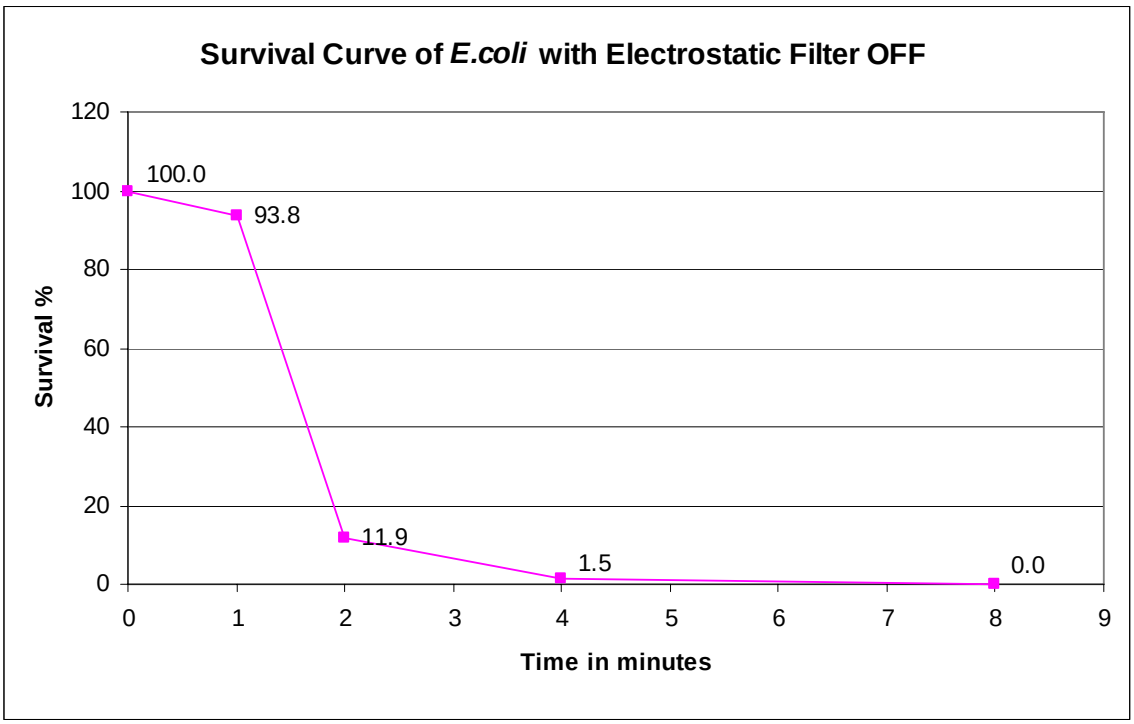

Fig 15: Survival curve graph of *E.coli* with electrostatic filter OFF.

Table 10: Viable *E.coli* count with electrostatic filter ON

Time of Exposure to the plasma in minutes	Colonies/Colony forming Units – CFU	Survival percentage (%)
0	412	100
1	369	89.56
2	78	18.93
4	74	17.96
8	18	4.37

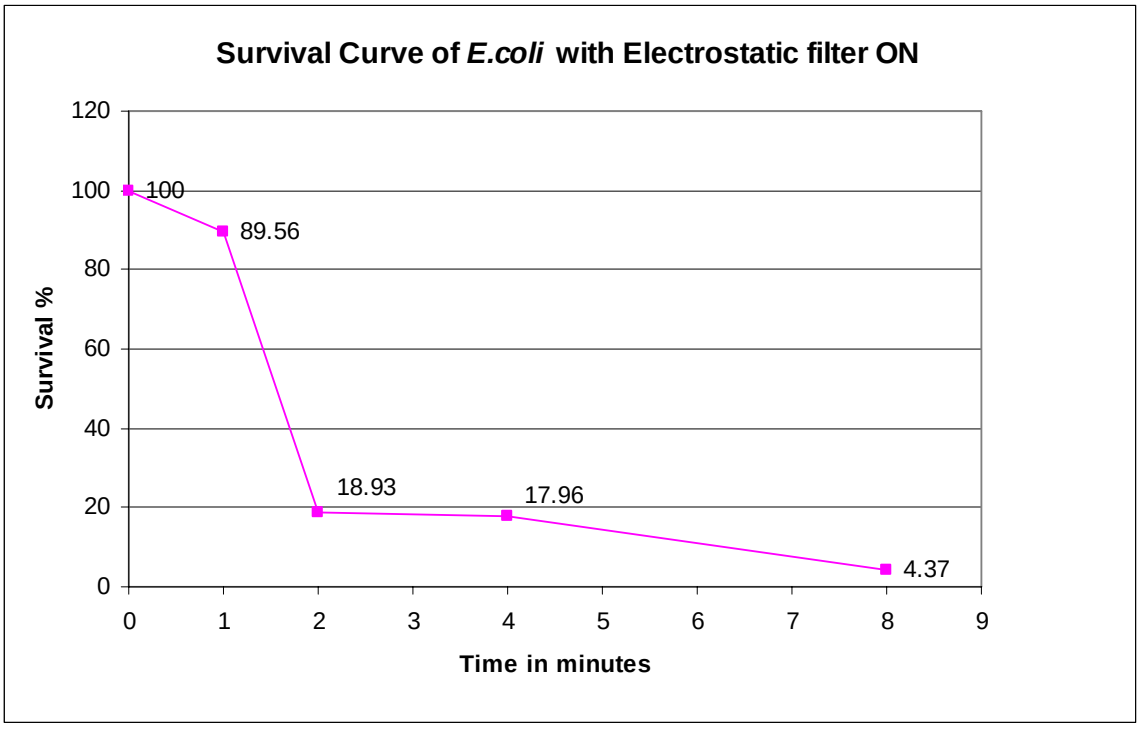

Fig 16: Survival curve graph of *E.coli* with electrostatic filter ON.

Note: Increase in sterilization times can be observed, lack of charged particles.

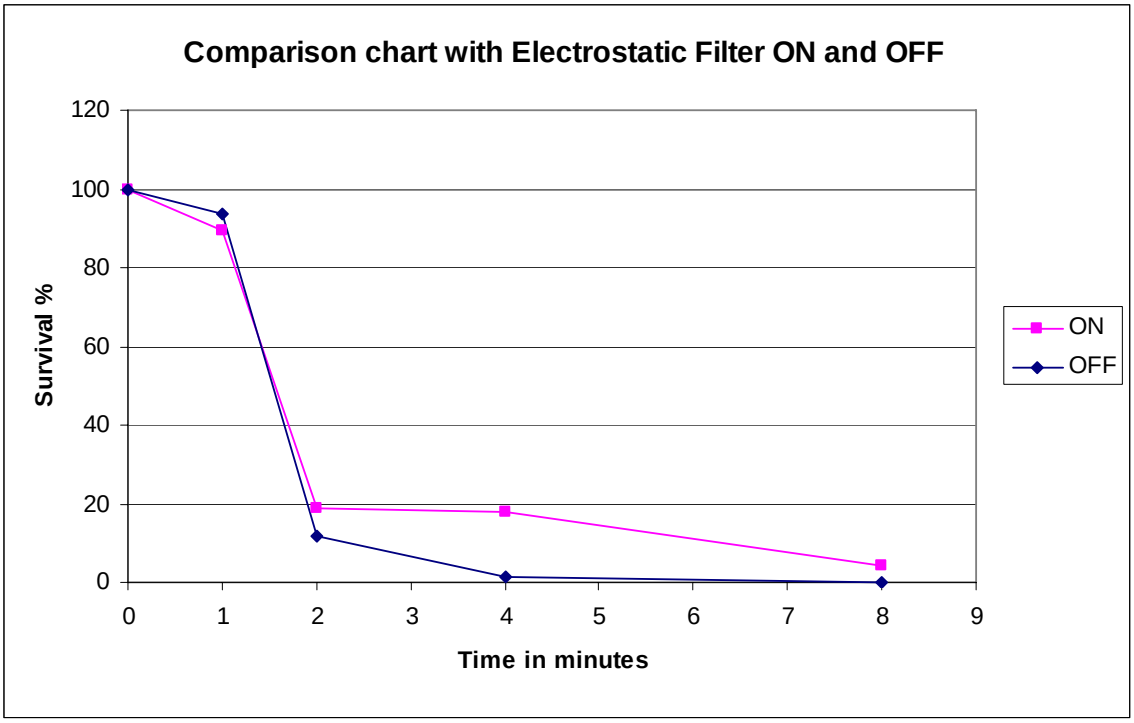

Fig 17: Comparison chart for electrostatic filters ON and OFF. The graph represents a comparison chart of the survival rates of *E.coli* after being exposed to the plasma with and without the electrostatic filter.

The results from these two experiments are easily comprehendible when presented as a comparison chart – shown in the next page. The objective of the electrostatic filter test is to reveal the influence of charge species in the sterilization of the bacteria. When the electrostatic filter is ON (when the electrodes are supplied with power) it sucks up the charged particles in the plasma. From the comparison chart it is evident that when the filter is ON the sterilization times are longer than when it is OFF, meaning that the charged particles contribute to the debilitation of the microorganisms,

however little it maybe. The observation proves beyond doubt that charged species if utilized efficiently can prove to be a significant component in plasma sterilization.

3.6 Hydrogen Peroxide Test Results

The use of hydrogen peroxide, a sterilant itself as a moistening agent in the ceramic has proved to be a major discovery. The results are tabulated in *Tables 11 and 12*. Two concentrations were used to detect the relation between the amount of hydrogen peroxide and sterilization. The concept is to generate more oxygen compounds in the discharge and study their effect. The sterilization times are very fast. The survival curves are presented in the Figures 18 and 19.

Table 11: Viable *E.coli* count with 3% H_2O_2. It shows the number of viable E.coli after exposure to atmospheric pressure air plasma in the presence of a 3% aqueous solution of hydrogen peroxide as a moistening agent.

Time of Exposure to the plasma in seconds	Colonies/Colony forming Units – CFU	Survival percentage (%)
0	406	100
30	57	14.04
60	8	1.97
120	0	0.00
240	0	0.00

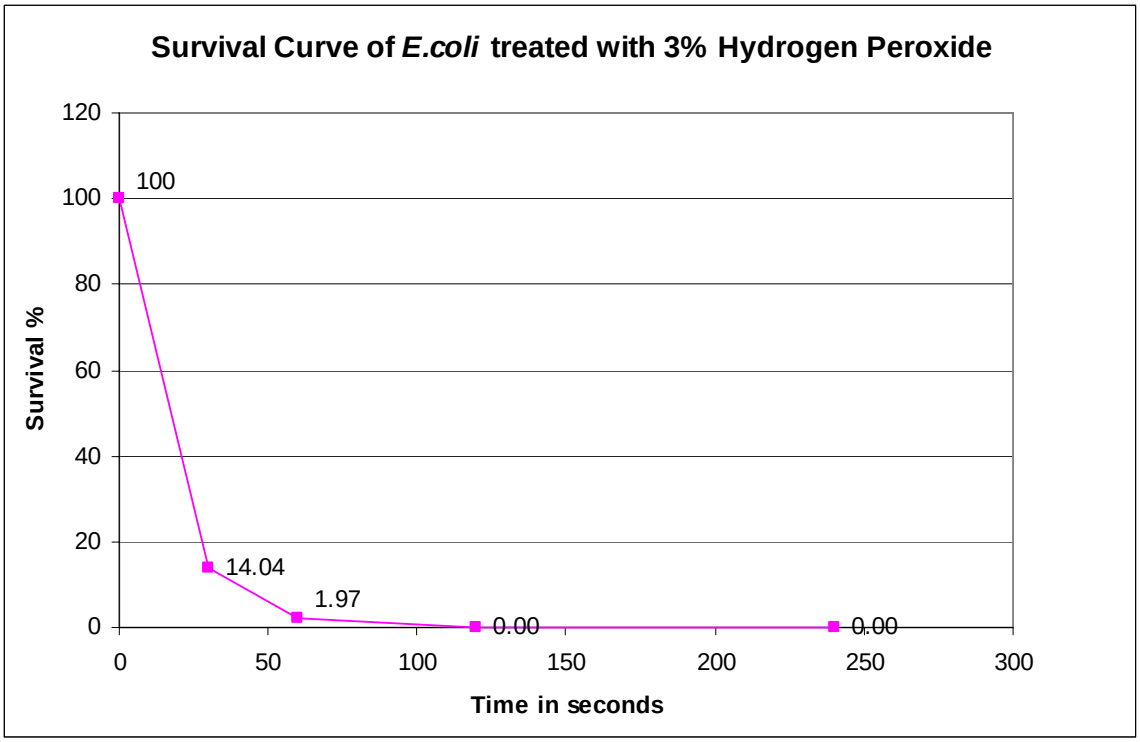

Fig 18: Survival curve graph of *E.coli* with 3% H_2O_2.

Table 12: Viable *E.coli* count with 30% H_2O_2. It shows the number of viable *E.coli* after exposure to air plasma in the presence of a 30% hydrogen peroxide.

Time of Exposure to the plasma in seconds	Colonies/Colony forming Units – CFU	Survival percentage (%)
0	411	100
30	14	3.41
60	0	0.00
120	0	0.00
240	0	0.00

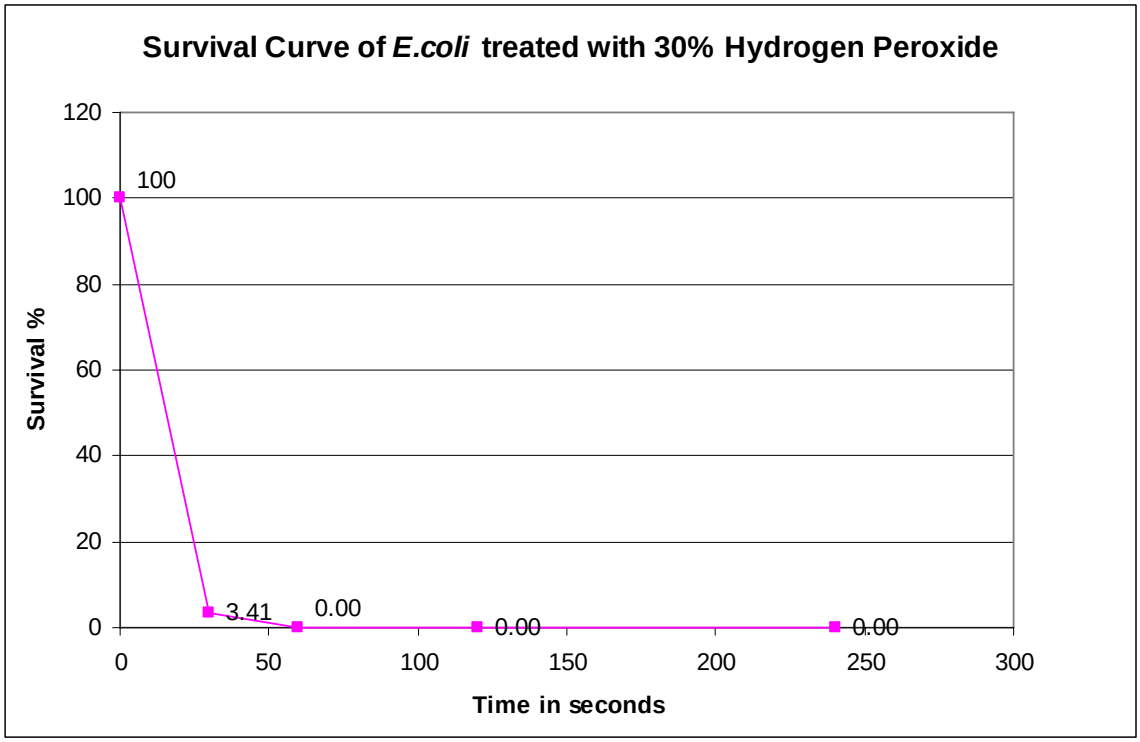

Fig 19: Survival curve graph of *E.coli* with 30% H_2O_2.

It is unmistakably proven from the results that the presence of hydrogen Peroxide reduces the times required for sterilization to a great extent. This could be due to the presence of more oxygen compounds, possibly meaning increased amounts of ozone or OH compounds. It is worthy to note that more the concentration, better the sterilization. In both cases (3%, 30% hydrogen peroxide) the times required for absolute zero sterilization is below 2 minutes, which is remarkable. Effectual sterilizations are achieved as low as 30 seconds. The most remarkable fact is that, the use of hydrogen peroxide as a moistening agent in the ceramic not only debilitates the microorganism completely but it alters/affects the growth medium in the pour plates in such a way that the medium is no more capable of supplying nutrients to the bacteria (*E.coli*), arising the possibility of the

deposition of the hydrogen peroxide vapors in the LB (*Luria Bertani*) medium and rendering it worthless for further growth of bacteria.

The comparison chart depicted in *Figure 20* further emphasizes the effectiveness of using hydrogen peroxide as moistening agent instead of water. The times required for sterilization are reduced 8 times when using Hydrogen Peroxide instead of water. Two photographs are presented in the next page as *Figures 21 and 22* show the effects of the plasma discharge with 3% H_2O_2 on the bacteria.

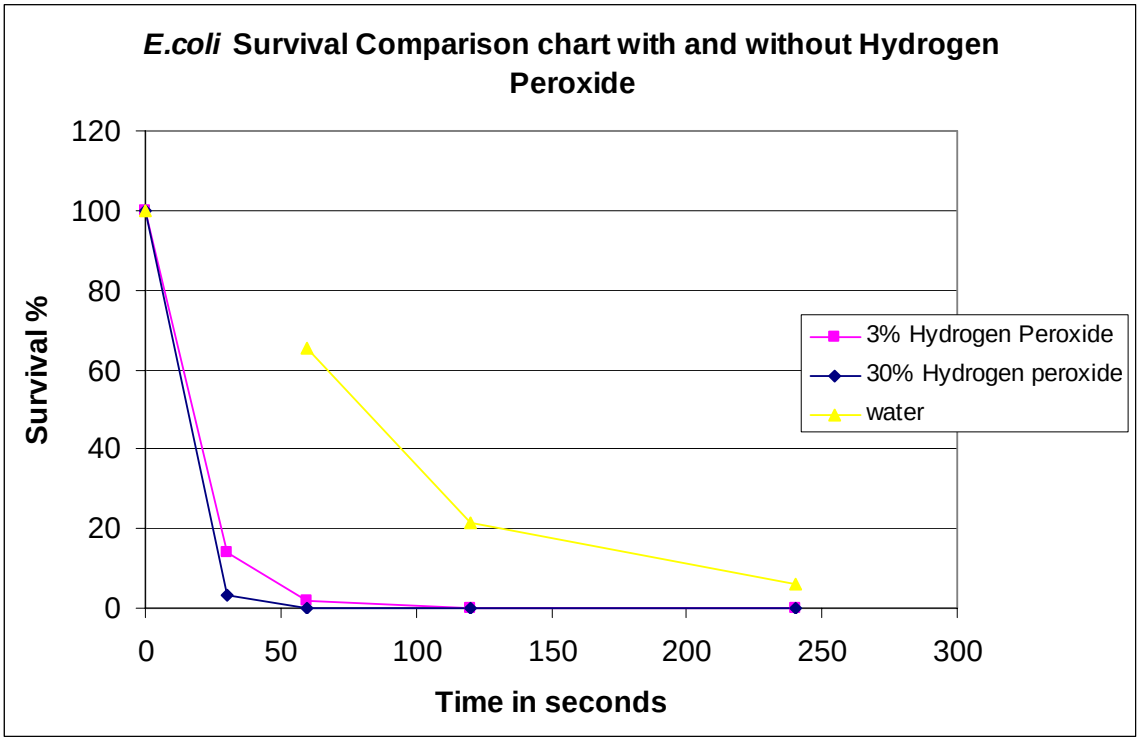

Fig 20: Comparison chart for 3%, 30% H_2O_2 and water.

Note: The effectiveness of Hydrogen Peroxide is clearly visible

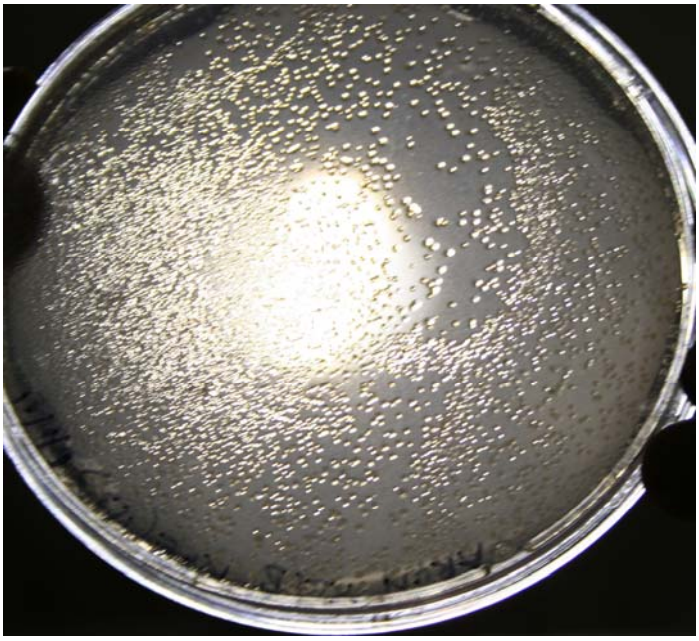

Fig 21: Photograph of the Petri Dish with the unexposed bacteria.

Fig 22: Photograph of the same Petri dish after exposure to the plasma.

This used water as the moistening agent.

Note: Reduction in the number of bacteria.

3.7 Bacteriophage (Lambda Phage) Results:

This test was conducted to test the efficiency of the sterilizer when it comes to viruses, because of the fact that viruses are far more resilient than the bacteria. The contributing factors to the survival of the bacteriophage were adjusted so that a humanly countable number of viruses were present before exposure to the plasma. In this specific case the factors were tuned to have around 150 plaques or Plaque Forming Units (PFU) which is the terminology pertaining to bacteriophages, comparable to Colony Forming Units (CFU) in bacteria. Again in this experiment we wanted to test the effects of using Hydrogen peroxide and so, two tests were performed on the bacteriophage, one with water as moistening agent *(Table 13 and Figure 23)* and the other with Hydrogen Peroxide *(Table 14 and Figure 24)*. *Figure 25* is a comparison chart of the two.

Table 13: Viable *plaque-phage* count without H_2O_2. The table shows the number of plaques (PFU's) after exposure to plasma in the presence of water.

Time of Exposure to the plasma in seconds	Plaques/Plaque forming Units – PFU	Survival percentage (%)
0	147	100
30	107	72.79
60	54	36.74
120	13	8.84
240	3	1.85
480	0	2.04

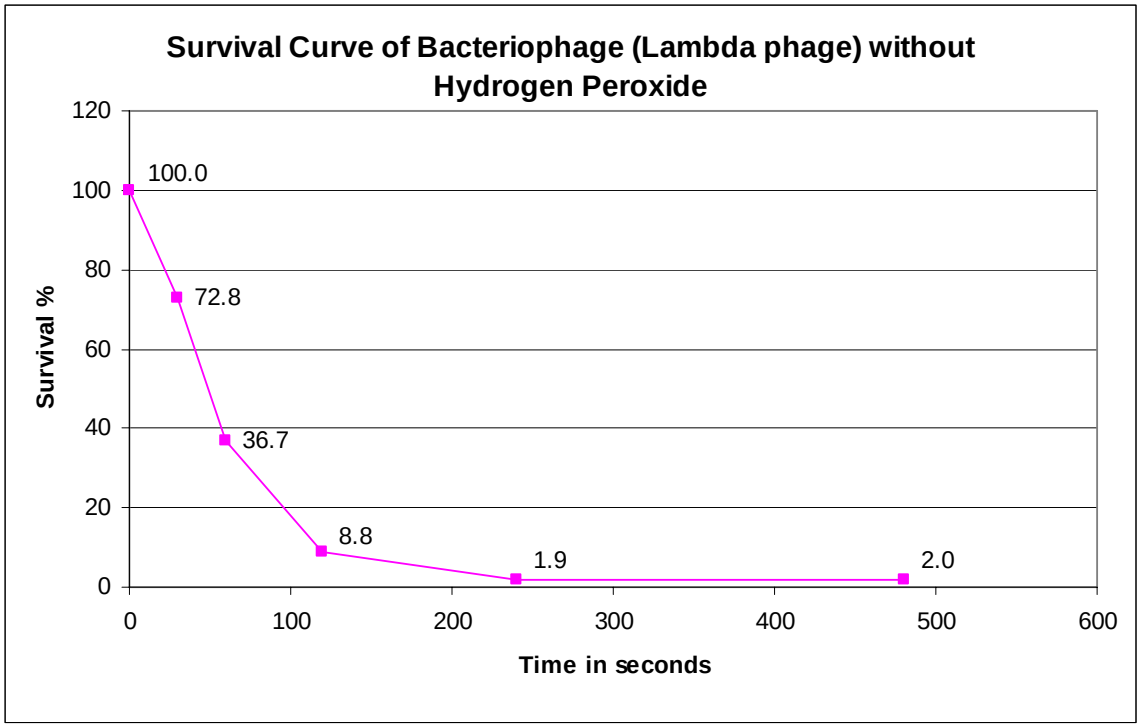

Fig 23: Survival curve graph for phage without H_2O_2.

Table 14: Viable *plaque-phage* count with H_2O_2. This table shows the count of viable PFU after exposure to plasma in the presence of 30% Hydrogen Peroxide.

Time of Exposure to the plasma in seconds	Plaques/Plaque forming Units – PFU	Survival percentage (%)
0	159	100
30	26	16.35
60	7	4.4
120	0	0
240	0	0
480	0	0

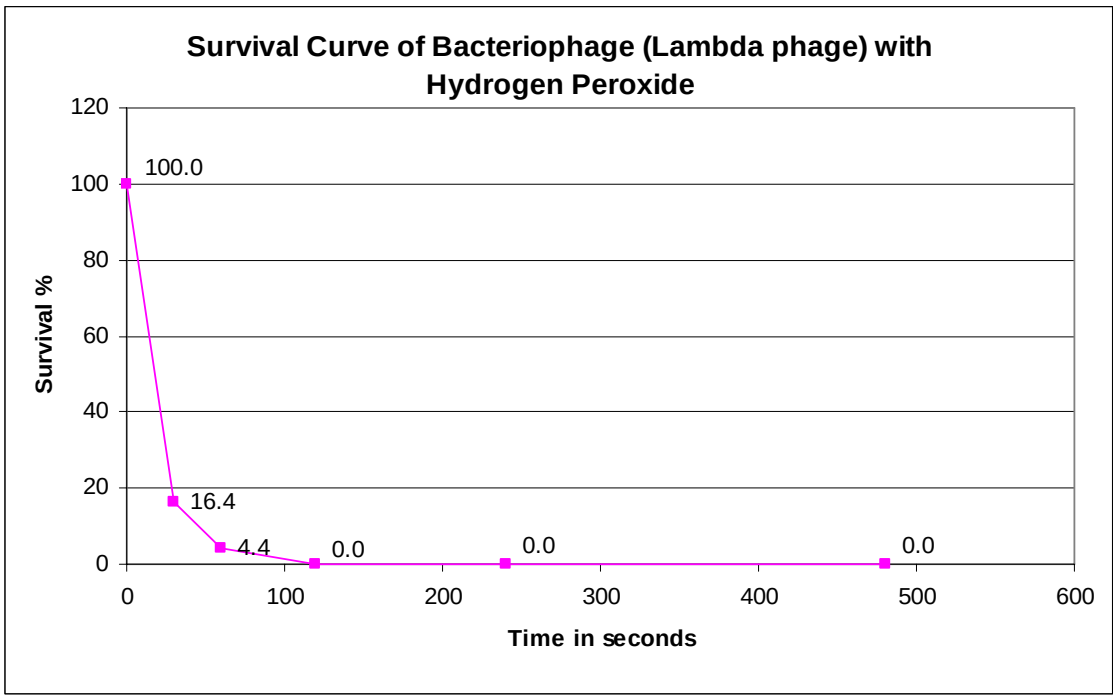

Fig 24: Survival curve graph for phage with H_2O_2.

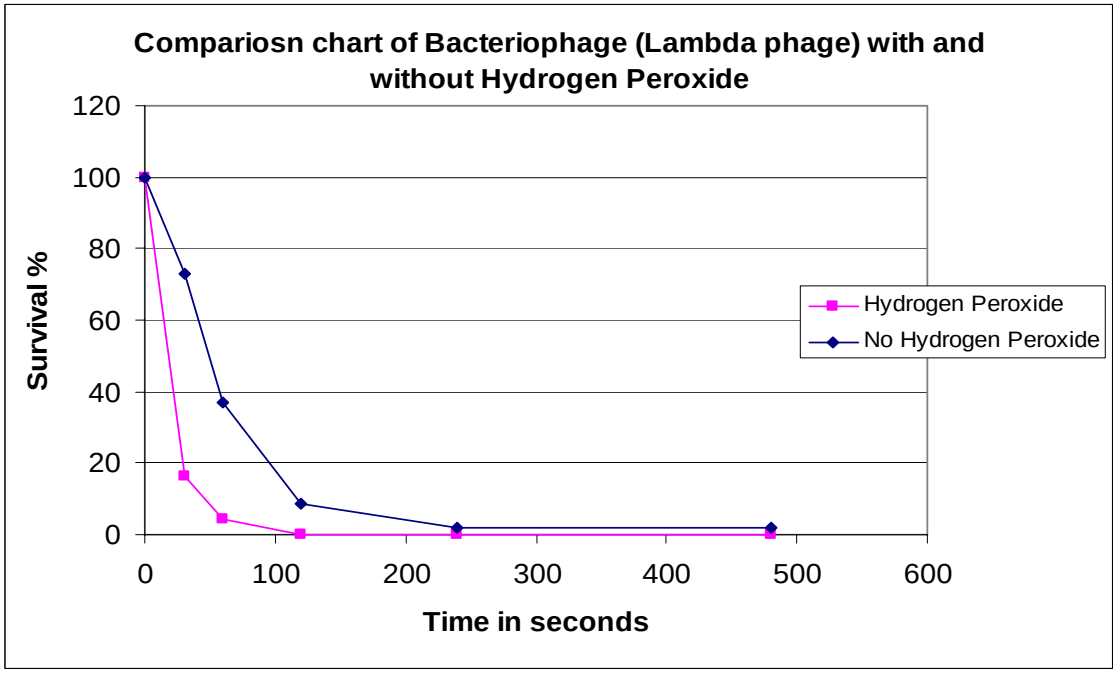

Fig 25: Comparison chart of phage survival with and without H_2O_2.

The plasma sterilizer was able to sterilize the bacteriophage effectively verifying its capacity to sterilize the more resilient virus and offering a comprehensive understanding of it being implemented as a sterilizer for treating a wide range of microorganisms. As expected, the use of Hydrogen Peroxide again proved to be more effective than water, whose comparison chart is presented in the next page. Two photographs showing effects of plasma sterilization on the Bacteriophage taken in the UT Microwave and Plasma Laboratory are presented in the next page as *Figures 26 and 27*.

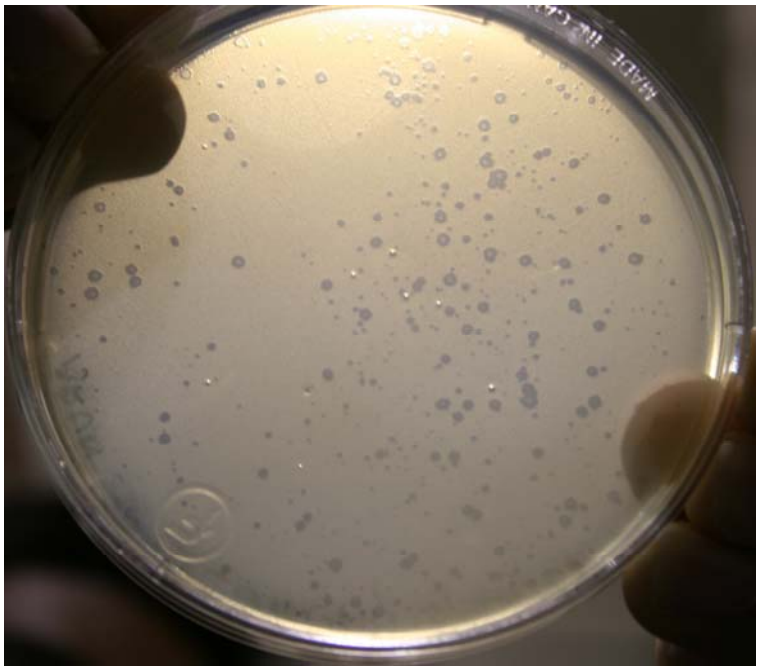

Fig 26: Photograph of the Petri Dish with the unexposed bacteriophage.

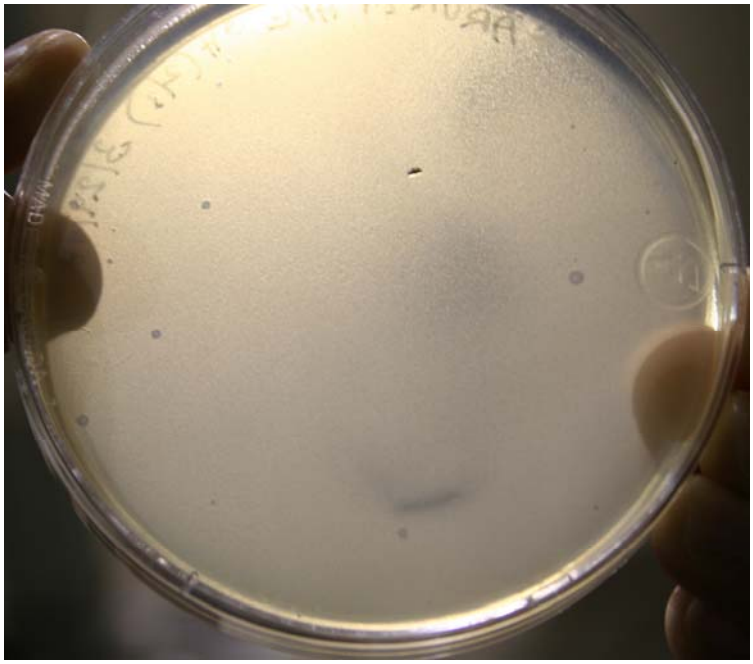

Fig 27: Photograph of the same Petri dish after exposure to the plasma.

Note: Reduction in plaque count

Chapter 4

Conclusions and Future Prospects

Some of the characteristic advantages and accomplishments of the discharge used in this research, future prospects and certain applications are enlisted in this chapter.

The ability of this discharge in generating gaseous discharges in large volumes and more importantly at atmospheric pressure renders them practical for various industrial applications meaning that they eliminate the need for expensive, inefficient vacuum systems typical to plasma discharges. The temperature in the discharge is comparable to room temperature, which further stretches its application boundaries.

The plasma decontaminator does not use a RF power supply which is most common power supply for atmospheric pressure gaseous discharges as of now which means that this discharge can be very cost efficient and less hazardous to operate. The non RF-power supply eliminates problems relating to stray electro magnetic radiation, which has a negative impact on operators (human beings). It also eliminates the need for expensive radiation protection equipments for sensitive electronics. The plasma reactor uses both AC and pulsed DC power supplies, which makes it more versatile and easy to use.

Since the discharge is electrically activated, there are no toxic components in the discharge. If at all there are any, they are recombined into air as soon as the power is turned off.

It can be operated in both active and passive exposure modes. The energy efficient passive exposure mode has wide ranging applications starting from air purifiers to hand sanitizers.

It has a combinational decontamination effect, meaning that there is not just one component that causes the sterilization. The experiments conducted in this research have revealed the presence ozone, charged particles, and other oxygen compounds in the discharge that play a role in the decontamination. Different types of working gases can be used, which is one of the most significant advantages. It does require a specific working gas and it can work in air, which certainly is the least expensive gas of all. In all cases the sterilization has been effective.

From subsequent studies it has been found that the use of hydrogen peroxide significantly decreases the total time and power required to accomplish sterilization. It not only sterilizes the microorganisms but also deprives the nutrition from the growth media, whereby avoiding further microbial proliferation for days after treatment. This effect is speculated to occur because of the deposition of the Hydrogen Peroxide Vapor (HPV) on the Petri dishes. In addition, the use of hydrogen peroxide also allows sterilization to occur in different types of packaging material. Since the hydrogen peroxide is decomposed into non-toxic products during the plasma treatment, no additional steps are required to remove residual hydrogen peroxide from the sterilized object or its packaging prior to handling of the material itself.

The plasma decontaminator can be used to sterilize a wide range of materials and different types of microorganisms on a surface and in air. Its capacity to sterilize resilient viruses (shown in this research) is an apt example for this claim.

Future prospects of this research possess extensive margins. From an educational research point of view, a few things that can be done are mentioned in this passage. Primarily, the exact influence of each of the constituents of the plasma (charged species, active species, OH radical, ozone, etc.) contributing to the debilitation can be observed with extensive chemical analysis. Secondly, from a biological point of view, the exact inactivation principle of the microorganism caused by the plasma could be analyzed.

In addition, the sterilizer could be tested with spores - bacterial or viral, which are more resilient forms of microorganisms than the virus itself. It is worth mentioning that the majority of sterilization techniques commonly use a specific spore for testing the process efficiency called *Geobacillus Stearothermophilus.* This microorganism is set as a standard because of the fact that it is not harmful to human beings and it is extremely resistant to many sterilization processes, including steam sterilization (extreme heat). This testing would ratify the potential of the plasma discharge being transformed into a large-scale commercial sterilizer.

The applications of the sterilizer are innumerable, since it is extremely difficult to list all of them only a selected few are listed. These include some major applications areas like medicine, military and hygiene. Medical equipment sterilization - without the toxic effects of Ethylene Oxide (EtO), Military applications – in chemical and biological warfare prevention, Commercial Hand sanitizers which could be used in conjunction with currently existing hand dryers, Surface treatments – to modify fabrics and many more.

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Vita

Eric Pius Pradeep was born in Coimbatore, India. He received his Bachelors (B.E.) degree in Electrical and Electronics Engineering from the University of Madras, India in 2003. Apart from academic excellence, he has won many laurels in athletics and soccer in his college years.

After working as an intern for an electrical engineering company in India for a little less than a year, he set in motion to pursue an advanced degree in engineering in the United States. He joined the Masters program in Engineering Sciences at University of Tennessee (UT), Knoxville in August 2004. He started work as a Graduate Research Assistant in the Microwave and Plasma Laboratory at the same time and has been working there ever since. He has three recent publications in his field of research.

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