# UTILIZATION OF DOUBLE DIELECTRIC BARRIER DISCHARGE (DBD) PLASMA REACTOR IN THE DESTRUCTION OF ESCHERICHIA COLI AND BACILLUS SUBTILIS

By

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1998

Submitted to the Faculty of the Graduate college of the Oklahoma State University In partial fulfillment of the requirements for the Degree of MASTER OF SCIENCE May, 2005

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#### ACKNOWLEDGEMENTS

I sincerely thank my major advisor Dr. John Veenstra, for his guidance, timely support and concern and undying efforts throughout the entire duration to bring out the best out of this project and my thesis. I also wish to thank the other members of my committee Dr. Arland H. Johannes and Dr. Gregory Wilber for their technical assistance. I'm also indebted to Dr. Gregory D. Holland for constructing my reactor, organizing my data and having helped me in my literature search.

The School of Civil and Environmental Engineering, staff and faculty alike deserve special mention for the continual assistance and financial support that I received throughout the entire period of my education here. Special thanks to Vijay Kalpathi for working with me shoulder to shoulder in conducting experiments, Vijai Krishnah Elango for teaching me how to use the GC, Rahul Chalke, Kirubhakarn Vyravan, Arun Kumar Venkateshwaran for helping in literature search, and my other friends and roomates for all their patience.

Words can not express the gratitude that I have for my parents, Mr. Paneerselvam. S and Mrs.Yasotharadevi. P for all the love, care, encouragement, and the efforts taken to ensure that I received the best at all times, immaterial of the costs involved.

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#### **Chapter I**

#### Introduction

#### **Indoor Air Quality (IAQ)**

After the worldwide energy crisis in 1973, the increase in the cost of energy has resulted in the improved construction and retrofitting of homes and commercial buildings to achieve energy conservation (Hess-Kosa, 2002). This has resulted in the reduction of infiltration of fresh air (which is cost effective and is an energy-saving scheme widely used). This has led to a large portion of the population in the U.S living in tightly sealed structures, recirculating about 80 to 90% of the indoor air to economize on energy (Hines et al., 1993).

In older, less efficient homes the air exchange rate was two changes per hour, which diluted and cleaned the indoor air contaminants. Newer buildings have an exchange rate of 0.2 to 0.3 air changes per hour, while energy efficient office buildings have air exchange rates of 0.29 to 1.73 air changes per hour. As a result of this low recirculation indoor pollutant levels have been reported to be about 100 times the outdoor pollutant levels. The Environmental Protection Agency (EPA) has ranked indoor air pollution among the top four environmental risks in America (Hess-Kosa, 2002).

#### **Indoor Air Contaminants**

According to the EPA and Consumer Product Safety Commission (CPSC), in A Guide to Indoor Air Quality (Office of Air and Radiation, 1993), Total Exposure Assessment Methodology (TEAM) studies revealed that there were about a dozen common organic pollutants in levels two to five times higher inside homes than outside, irrespective of whether the home was located in a rural or industrialized area.

The listed indoor air pollutants are:

- 1) Radon,
- 2) Volatile Organic Chemicals (VOCs),
- 3) Bioaerosols,
- 4)  $CO_x$ ,  $NO_x$ , and
- 5) Respirable articles (including Tobacco smoke).

Biological air pollutants are the main concern of this research. Biological air pollutants include bacteria, bacterial spores, fungi, protozoa, microbial products originating from ventilation systems and pollen. Biological air contaminants have the potential to cause a severe health condition called hypersensitivity pneumonitis, and other disorders including humidifier fever, allergic rhinitis, conjunctivitis, as well as diseases like tuberculosis, diphtheria and allergic reactions (Maus et al., 1997; NIOSH, 1987). NIOSH included microbial contamination as the third in the list of three major problems affecting IAQ (NIOSH, January 1987). In recent years, fear of biological warfare and the outbreak of SARS (Severe Acute Respiratory Syndrome) (among other factors) has escalated the need for novel technologies for microbial decontamination of possible susceptible attack locations like federal government buildings, public transportations systems and military

installations (Birmingham et al., 2000). Double dielectric barrier discharge (DBD) plasma reactor was considered for the destruction of Escherichia *Coli (E. coli)* and *Bacillus Subtilis (B. subtilis)* as it has been used widely for destruction of microorganisms in studies by various authors.

#### **Objectives**

DBD will be examined and compared to conventional methods like ultraviolet (UV) radiation, high efficiency particulate air (HEPA) filtration, and disinfection with ozone for the destruction of *Escherichia Coli* (*E. coli*) and *Bacillus subtilis* (*B. subtilis*). The three main objectives of this study are:

- To determine the efficiency of the DBD reactor for destruction of *E. coli* and *B. subtilis* in bioaerosol-contaminated air under various operating conditions.
- To determine the efficiency of the DBD reactor for destruction of *E. coli* and *B. subtilis* in bioaerosol-contaminated nitrogen gas under various operating conditions.
- 3) To determine the efficiency of the DBD reactor when particulates are introduced into the bioaerosol-air stream. In other words the objective is to determine the destruction efficiency the DBD reactor when a potential (in the form of particulates) was provided for the microbes to survive.

#### **Chapter II**

#### **Literature Review**

#### Methods Available To Sterilize Air

Sterilization is the physical or chemical process that is used to destroy or eliminate all forms of life, especially microorganisms (Moisan et al., 2002). According to S.S.Block, in Encyclopedia of Microbiology, sterilization is "any process or procedure designed to entirely eliminate microorganisms from a material or medium". Sterilization has also been defined as inactivation of microorganisms to prevent infection (Akitsu et al., 2004). For the airborne microorganisms to be infectious they need to be viable, but this condition is not valid for organisms causing allergic effects. Hence the technology used for sterilization must be able to make the microorganisms nonviable (Maus et al., 1997).

#### A) Air Filtration

Mechanical filtration (air filtration) is the most predominant technology in use to control airborne particles indoors (Jaisinghani, 1998). High Efficiency Particulate Air (HEPA) filters are considered to be the best in commercial filtration of airflows from heating, ventilating and air conditioning systems (HVAC), these filters are available in the form of unglazed porcelain, asbestos or sintered glass (Laroussi, 1996). These filters are also made of glass fiber filters and have the ability to capture particles of 0.3 µm diameter, with an efficiency of 99% (Kelly-Wintenberg et al., 2000).

The principle at work in a HEPA filter is not to confine the particle/pathogen but to make the air flow through the convoluted overlapping threads so that microorganisms, not possessing the same inertia as the air molecules, are left behind. Also the fibers used are adhesive and hence the particles/microorganisms are retained (Lesavoy et al.).

Though fibrous filters are inexpensive and can be utilized without replacement over a long period of time (Maus et al., 1997). They are not free form disadvantages like growth of fungi and mold on the surface of the filters, thereby creating a potential source for allergies and diseases (Salie et al., 1995). This phenomenon is considered responsible for the Legionnaire's outbreak at the veterans convention in Philadelphia in 1976 (Jaisinghani, 1998). Though bacteria cannot grow on the clean glass filter fiber media used in HEPA filters, under normal humidity conditions, it can thrive on the dust that accumulates on the filters over a period of time and consume the accumulated dirt for their growth (Jaisinghani, 1998).

Jaisinghani et al. (1996) performed a set of experiments with clean 6"x6"x2" deep glass mini pleat filters. These filters were first exposed to an *E. coli* aerosol followed by pure air which was allowed to flow through the filter for 4 hours, with air temperature maintained at  $70^\circ \pm 5^\circ$  F with a relative humidity (RH) of  $50\% \pm 5\%$ . The filter was cut, then the bacteria was extracted and placed on a medium and then incubated for 24 hours. The results showed very few of the *E. coli* survived. Another similar test was performed, but in this test 1g of colloidal kaolin (contaminant) was added to the *E. coli* solution that was to be aerosolized. The recovery of *E. coli* was about  $10^4$ - $10^5$  Colony Forming Units (CFU)/square inch of the filter media. Similar work was done with *S. epidermidis* and the results of these tests show that common bacteria can survive and grow on glass HEPA filters under normal temperature and RH conditions (Jaisinghani et al, 1996).

The disadvantages of air filtration suggest that air filtration/HEPA filters may not be suitable for control of microorganisms. Filtration can not be stated outright as a sterilization technique as it doesn't remove all microorganisms (Laroussi, June 1996). A possible way to handle this could be by using an electric discharge in association with HEPA filter to prevent microbe multiplication on the filter and to prevent recontamination of airflow (Kelly-Wintenberg, 2000).

#### **B)** Ozone

Ozone is triatomic oxygen, represented as  $O_3$ . It's a bluish explosive gas or blue liquid found naturally in the atmosphere at sea level (at very low concentrations). Ozone is a very reactive oxidizing agent with a short half-life, after which it degrades back to its stable state of diatomic oxygen (National Organic Standards Technical Advisory Panel Review, August 2002). Ozone can reach all the corners of a room and hence is considered better than UV radiation and HEPA filters as it can reach more area (LAM). Kowalski et al. (1998) placed petri dishes with *E. coli* in an ozonation chamber of approximately 72 liters volume. The temperature inside the chamber was maintained at 23 °C – 24 °C, and relative humidity at 18 – 20%. Destruction efficiency higher than

6

99.99% was reported for ozone concentration in the range of 300 - 631 ppm and treatment time of 15 seconds.

Ozone is effective against microorganisms as it interferes with cellular respiration (Laroussi, 2003). When treated with ozone, ozone attacks the cell surface, thereby altering the permeability of the membrane. The main attack by ozone is on the double bond of the unsaturated lipids in the cell membrane. Tests conducted on *E. coli* revealed that ozone attacked the primary structure of nucleic acids only after they had been released by lysis. This led to the conclusion that ozone doesn't penetrate the cells, but acts on the surface first and then on the nucleic acids (Scott et al., 1962).

Komanapalli et al. (1996) did further work on *E. coli* (K-12), as *E. coli* was considered to provide insights into ozone's mechanism with microorganisms. Ozone was found to be mutagenic and able to degrade DNA in different strains of *E. coli*.

Another study by Komanapalli et al. (1998) was aimed at establishing a time-inactivation relationship. In this study they established that the viability of *E. coli* was not affected in the first 5 minutes of treatment. After 10 minutes of treatment the *E. coli* was reduced to  $1/100^{\text{th}}$  of the initial concentration, and after 40 minutes of ozone exposure *E. coli* was reduced by a factor of  $10^5$ .

Even though ozone has high destruction efficiency of airborne microorganisms the United States Environmental Protection Agency (USEPA/EPA) does not approve the use of ozone for indoor air pollution control. The reason stated by EPA on its website is as follows:

"Available scientific evidence shows that at concentrations that do not exceed public health standards, ozone has little potential to remove indoor air contaminants." "If used at concentrations that do not exceed public health standards, ozone applied to indoor air does not effectively remove viruses, bacteria, mold, or other biological pollutants."

EPA's National Ambient Air Quality Standard (NAAQS) for ozone is a maximum 8-hour outdoor concentration of 0.08 ppm.

The reasons quoted by EPA, for making the above statement, are:

- Ozone concentration should be 5-10 times higher than public health standards to inhibit growth of microorganisms.
- Even at high levels of ozone microbes embedded in porous material like duct lining or ceiling tiles may not be affected at all.

#### C) UV Irradiation / Ultraviolet Germicidal Irradiation (UVGI)

A fraction of the electromagnetic radiation in the range of 100nm – 400 nm is known as Ultraviolet (UV) radiation and this radiation is known to inactivate biological pathogens by damaging the microorganisms DNA and other cell components beyond the ability to replicate (Lesavoy et al, 2004). Dr.Niels Ryberg Finsen was the first (1903) to recognize and use the bactericidal effects of sun in treating infectious skin disease (Lesavoy et al). UVC (UV short range band of 200 nm to 280 nm) has been widely used for germicidal applications. 265 nm has been proven to give maximum germicidal effectiveness. To achieve 90% destruction of *B. subtilis* and *E. coli* at 253.7 nm, the energy required is  $12,000\mu$ W/cm<sup>2</sup> and  $3,000\mu$ W/cm<sup>2</sup>, respectively (Scheir et al., 1996). After World War II the use of UVC to decontaminate upper room air by directing a UV beam at the ceiling became more prevalent (Scheir et al., 1996).

The susceptibility range for UVGI is from *Sreptococcus* species, the most susceptible, to mycobacterium tuberculosis (MTb) which is moderately susceptible. The least susceptibility is exhibited by spore forms of bacteria (Brickner et al., 2003).

Salie et al (1995) evaluated the germicidal action of UV light positioned in a modified hollow ceiling fan blade and achieved reductions of 72.8, 3.8 and 8.6% for *E. coli*, *M. luteus*, and *B. subtilis* respectively, for a reaction time of 26 msec. According to Laroussi (2002) UV radiation induces the formation of thymine dimers in the DNA of the bacteria, thereby inhibiting the ability to replicate.

Due to concerns of UV radiation affecting the occupants in the room, the UV lamps will have to be wall or ceiling mounted with appropriate shielding (Jaisinghani et al., 1998). Another demerit of UV is that it can only disinfect the air that is close to the lamp as UV light has limited penetration capacity (LAM).

#### **D)** Plasma-Based Sterilization

Various descriptions are used to identify plasma. Plasma is the fourth state of matter and could also be described as an ionic gas or a gaseous complex comprised of electrons, ions of either polarity, gas atoms, molecules in ground and excited state, and light quanta

occurring due to the application of an electric field. Plasmas may also be classified based on the method of generation as "cold", or Non-Thermal Plasma (NTP), if the gas temperature is at ordinary temperature and the electrons are at a higher temperature and "hot", or Thermal Plasmas (TP), in which there is Complete Thermal Equilibrium (CTE). In other words, the electron and gas temperatures are in equilibrium. (Venugopalan, 1971). Thus plasmas can be categorized as thermal plasma (high temperature plasma or equilibrium plasma) and non-thermal plasma (non-equilibrium plasma or cold plasma) based on their energy level, temperature and ionic density. The main application of NTP is in flue gas treatment, due to its low power requirement and its potential to induce physical and chemical reactions within gases at relatively low temperatures. In a NTP, electrons can reach temperatures in the range of 10,000 - 100,000 kelvin (1 - 10 eV) while the gas temperature remains at room temperature (Liu et al., 1998). NTP recently has received attention for its other applications like excimer-light source, surface modification of polymers, and biological and chemical decontamination of media (Laroussi, 2002).

Non-equilibrium plasmas are divided into five groups depending on the mechanism used for generation, pressure range and electrode geometry : (Eliasson and Kogelschatz, 1991b):

- a) The glow discharge,
- b) The corona discharge,
- c) The silent discharge
- d) The radio frequency (RF) discharge,

e) The microwave discharge.

Laroussi (2002) identified corona discharge, glow discharge at atmospheric pressure or one atmosphere uniform glow discharge plasma (OAUGDP), atmospheric-pressure plasma jet (APPJ) and resistive barrier discharge (RBD), which are variations of the above stated groups, as the most extensively used technologies for their germicidal effects.

#### a) The Glow Discharge

A glow discharge (Figure 1) is a low pressure discharge (less than 10 mbar) occurring usually in between flat electrodes. Glow discharge plasmas are not used for industrial production of chemicals due to low pressure operation which in turn results in low mass flow, but they are widely used in the lighting industry (eg., neon bulbs) (Eliasson and Kogelschatz, 1991b). Figure 1 represents a glow discharge reactor used for catalyst preparation (Liu et al., 2002).

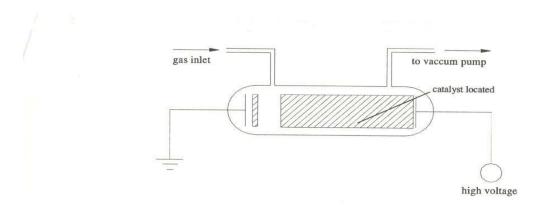


Figure 1 Glow Discharge Reactor (Liu et al., 2002)

#### b) The Corona Discharge

A corona discharge can be initiated at atmospheric pressure using inhomogeneous electrode geometries like a pointed electrode and a plane or a thin wire. The small radius of curvature at the top of the electrode results in the production of the high electrical field required to ionize the neutral molecules. Corona discharge is utilized in places where small concentrations of charged species are sufficient, Examples are electrostatic precipitators and copying machines. Other uses are large scale flue gas treatment, high-speed printout devices, dry-ore separation systems, radiation detectors and surface treatment of polymers (Eliasson and Kogelschatz, 1991b).

The first recorded use of plasma technology for inactivation of microorganisms was by Siemens, who suggested that corona discharge could be used to generate ozone to disinfect water supplies (Laroussi, 2002). Garate et al (2003) destroyed concentrations of up to  $10^{10}$  mL of *E. coli* and spores of *B. subtilis* in less than 15 minutes by using an "Enhanced Corona Discharge" shown in Figure 2 (Laroussi, 2002).

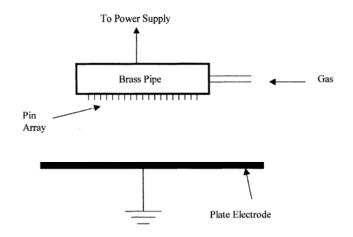


Figure 2 Configuration of Enhanced Corona Discharge (Laroussi, 2002)

#### c) The Silent Discharge

The silent discharge combines large volume excitation of the glow discharge and the high pressure of the corona discharge to produce plasma. The silent discharge (Figure 3) typically consists of a dielectric layer covering at least one of the electrodes which explains the terms "dielectric barrier discharge" or "barrier discharge" used to refer the silent discharge. The dielectric layer must have a high dielectric constant, usually Pyrex, quartz or ceramics are used as the dielectric layer. (Cal and Schleup, 2001)

Once a voltage (about 8 to 30 kV) (Cal and Schleup, 2001) is applied across the electrodes with the dielectric barrier(s), a host of current filaments of short duration is formed, the life cycle of a filament undergoes the following three steps: (Eliasson and Kogelschatz, 1991a)

- 1) Formation of discharge or electrical breakdown
- 2) Movement of charge across the gap
- Simultaneous excitation of the molecules and atoms resulting in the initiation of reaction kinetics

The dielectric barrier accumulates the charges on itself once ionization occurs thereby limiting the amount of charge transported by a single micro discharge and discharges the micro discharge over the entire electrode. Breakdown in a silent discharge occurs at electron energies of 1 - 10 eV and this is the ideal range for breaking chemical bonds, and exciting atoms and molecules (Eliasson and Kogelschatz, 1991b).

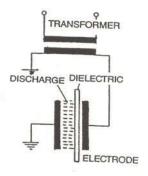


Figure 3 Silent Discharge Reactor (Chang, 2001)

The OAUGDP and RBD are both based on the principle of silent discharge with certain variations. Setup similar to Figure 4 (with an air gap) was used by Kelly-Wintenberg et al (1998) to inactivate *E. coli, S. aureus, B. subtilis,* and they reported 90% destruction of *E. coli* cells in five seconds Laroussi (2002) reported that using the DBD based diffuse-glow discharge a variety of gram negative, gram positive bacteria have been inactivated by many researchers.

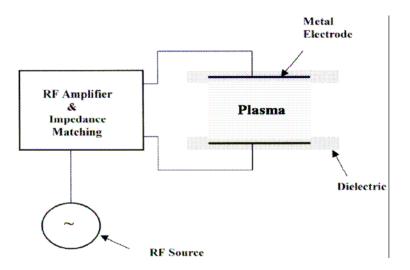


Figure 4 DBD-Based Diffuse Glow Discharge (Laroussi, 2002)

The RBD uses a high-resistivity material, instead of a dielectric material, to cover at least one of the electrodes as represented in Figure 5. This high-resistive material limits the discharge current and thereby limits arcing. Unlike the DBD, the RBD can use dc (direct current) power (or low frequency ac (alternating current), 60 Hz).

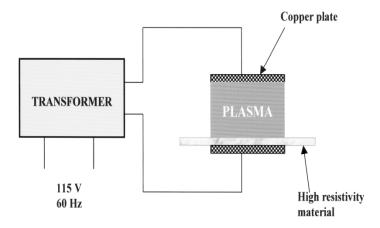


Figure 5 Resistive Barrier Discharge (Laroussi, 2002)

#### d) The Radio Frequency (RF) Discharge

The RF discharge (Figure 6) can perform well both at low and atmospheric pressures. RF discharges can be operated with the electrodes placed outside the reactor hence avoiding electrode erosion and contamination of plasma by the metal vapors. The RF discharge finds widespread use in labs to produce plasmas for optical emission spectroscopy and for plasma chemical investigations. Low-pressure RF discharges for etching are used extensively in semi-conductor manufacturing. (Eliasson and Kogelschatz, December 1991b)

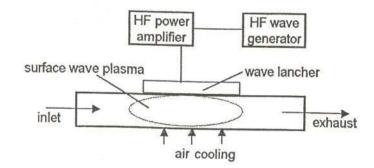


Figure 6 RF Reactor (Chang, 2001)

The APPJ (Figure 7) uses RF power (13.56 Hz) to excite the central electrode. The RF field accelerates the free electrons, which collide with background gas to produce various reactive species that exit the nozzle at very high velocity, the contaminated surface should be placed close to the nozzle to achieve decontamination (Laroussi, August 2002).

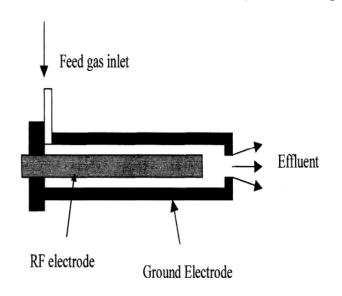


Figure 7 Atmospheric Pressure Plasma Jet (Laroussi, 2002)

#### e) The Microwave Discharge

Microwave discharge operates in a wide pressure range of 1 mbar to about atmospheric pressure. Microwave induced plasmas are created in a wavelength structure or resonant cavity as the wavelength of the electromagnetic field becomes comparable to the

dimensions of the discharge vessel, in the microwave region of (0.3 Hz - 10 GHz). The microwave discharge plasma (Figure 8) can produce a large volume of non-equilibrium plasma with reasonable homogeneity over a wide range of frequencies and pressures. The microwave plasma finds application in elemental analysis and lasing media.

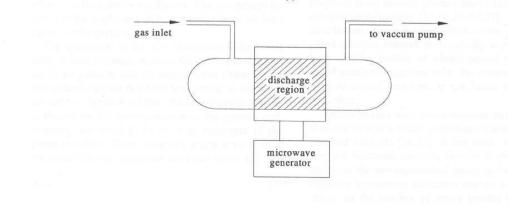


Figure 8 Microwave Reactor (Liu, 2002)

### Plasma Chemistry and Mechanisms

In a plasma reactor, ozone if formed due to the neutral particle conversions in a discharge zone and is formed according to the following reaction:

$$O + O_2 + M \to O_3 + M \tag{1}$$

Where,

M is the N<sub>2</sub> molecule

Ozone reverts to the original form of oxygen, in dry air, according to the following

reactions

$$O_3 + M \to O_2 + O + M \tag{2}$$

$$O_3 + H \to OH + O_2 \tag{3}$$

$$O_3 + OH \to HO_2 + O_2 \tag{4}$$

 $O_3 + HO_2 \to OH + 2O_2 \tag{5}$ 

If humid air is used another reaction that takes place along with equations (3), (4), (5) is:

$$OH + OH \to H_2O_2 \tag{6}$$

Reactions (1) - (6) are from Efremov et al (2000).

These radicals (OH, HO<sub>2</sub>, H<sub>2</sub>O<sub>2</sub>) have antiseptic action on the microorganisms (Efremov et al., 2000). Detailed reactions have been listed in Appendix A. Active species produced in an OAUGDP include ozone, monoatomic oxygen, free radicals like superoxide, hydroxyl, and nitric oxide, and ultraviolet photons (Kelly-Wintenberg et al., 1999). To determine if UV played a role in the destruction mechanism tests were conducted on microorganisms placed in sealed opaque bags (which do not permit UV) and on comparison with tests conducted on microorganisms exposed to air and it was concluded that UV photons are not the major antimicrobial active species as the results (with respect to time) were the same (Kelly-Wintenberg et al., 1999).

Mechanisms differ according to the pressure at which the plasma is operated. M.Moisan et al. (2002), focusing on low pressure plasmas ( $\leq 10$  torr) have suggested the following mechanism for the occurrence of biphasic or three phased survival curve (log no. of survivors Vs time treated) in plasma based sterilization of bacterial spores:

- 1) Destruction of genetic material by <u>UV radiation</u>.
- <u>Intrinsic photodesorption</u> resulting in atom by atom erosion of the microorganism. This desorption is a result of UV photons disrupting the chemical bonds of the microorganisms which results in the formation of volatile compounds (eg. CO and CH<sub>x</sub>).

3) Erosion by <u>etching</u>. In the process the reactive species (O, O<sub>3</sub>, metastable molecules) from the plasma is adsorbed on the microorganisms with which they undergo chemical reactions to form volatile compounds. This process could also be enhanced by UV photons.

The assumptions made were:

- UV photons and reactive species are both present throughout the inactivation process.
- 2) A, B and when existing mechanism C are active from beginning to end of the survival curve.
- 3) Ultimate inactivation of all spores is by UV photon irradiation.

The inactivation starts with UV photons destroying the DNA of microorganisms, this leads to the accumulation of cell debris forming a layer on top of the active spores or living cells, thereby shielding them from the UV. Now (as shown in Figure 9) photodesorption and etching occur due to the radicals and other active species present, volatilizing the debris opening the way for UV to reach the rest of the cells for inactivation.

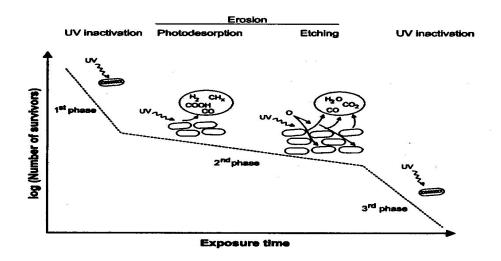


Figure 9 Three Phase Survival Curve (Moisan et al., 2002)

Laroussi et al.'s work (2002) agrees with Kelly-Wintenberg et al.'s (1999) suggesting that UV does not play a vital role in the inactivation of airborne microorganisms for highpressure plasma reactors. Montie et al. (2000) proposed three mechanisms for high pressure plasmas:

- 1) Lipid peroxidation due to attack of hydroxyl radicals on unsaturated fatty acids.
- 2) Oxidation of amino acids resulting in protein oxidation.
- 3) DNA oxidation due to reaction with oxygen radicals.

Experimental results have also shown that the discharges containing oxygen have strong germicidal effect due to the presence of oxygen based active species like atomic oxygen, metastable singlet oxygen and ozone (Laroussi, 2002).

Montie et al. (2000) and Laroussi et al. (1999) reported that *E. coli* underwent outer membrane rupture after short exposures (10-30s) to plasma, which was followed by leakage of their cytoplasm. Total cell fragmentation occurred for longer exposure times.

Mendis et al (2000) have proposed that cells are killed due to the accumulation of electric charge on the cell surface, this accumulation results in electrostatic stress and when it exceeds the cell's tensile strength, physical disruption of the cell membrane occurs. Mendis et al have also explained that for accumulation to take place the surface must have irregularity or regions of high local curvature. Therefore this explanation could suit only gram negative bacteria which have irregular surfaces.

Most of the work done by different authors has concluded that with plasma decontamination of microorganisms greater than 90% reduction in microorganisms can be achieved. Montie et al (2000) obtained 99.99% destruction of *E. coli* K12 (on polypropylene) for an exposure time of 24 seconds in a OAUGDP. Birmingham et al (2000) reported a deactivation of 99.9999% of the aerosolized *Bacillus globigi* (*B. globigi*) in tested with a corona reactor.

Nelson et al. (1989) treated *B. subtilis* placed on sterile polystyrene Costar 96 multiwell tissue culture tray in a plasma reactor. The power used was 50 and 200 watts, exposure time was 5, 30 and 60 minutes. The temperature of the culture was maintained at 25 °C, 100% inactivation of *B. subtilis* was achieved after exposure to helium and argon gas plasmas.

Kelly-Wintenberg et al (1999) used an OAUGDP and their results for *E. coli* seeded on glass, agar and polypropylene and *B. subtilis* embedded in paper strips were about 98.57% and 99.1% respectively. The exposure time for E. coli ranged from 30s to 5 min

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based on the surface it was seeded and the exposure time for B. subtilis was 5.5 min. They tested other microorganisms too and concluded that a bacterial virus was the most difficult to kill as it required 9 minutes to inactivate six logs.

Effremov et al (2000) achieved 99.9% destruction of *E. coli* with a glow discharge reactor operated at a discharge voltage of 4.5 kV and a current of 225mA. The pressure and temperature inside the discharge chamber were 0.65 atm and 15 °C respectively and the exposure time varied from 10 - 60 seconds.

The advantages of using a glow discharge plasma reactor at atmospheric pressure (OAUGDP) are (Kelly-Wintenberg et al., 1998):

- Kills microorganisms and spores, at room temperature, by a relatively simple, safe and fast process.
- 2) Doesn't require batch processing.
- Materials are not exposed to high temperatures and pressure like in steam sterilization, no toxic gases or high doses of radiation are used.
- 4) Kill spores and vegetative cells in seconds to minutes.

#### Nonculturable Bacteria

Viable bacteria are further divided into culturable and nonculturable. Culturable bacteria are those than can be grown in laboratory conditions, and Huang's (1996) testing counted only these bacteria. Nonculturable bacteria are those that are still viable but can not reproduce under laboratory conditions due to cell damage or stress (Jensen et al., 1998).

Nonculturable bacteria can be enumerated by green fluorescent protein (GFP) tagging followed by analysis on a flow cytometer (Lowder et al, 2000).

### **Chapter III**

### **Materials and Methods**

All the experiments necessary for this work were performed by Huang (1996) and Schroeder (1996) at the Oklahoma State University's Hazardous Reaction Laboratory. The entire experimental setup, except for the Variac and the sampling bomb were placed in a fume hood, to isolate the high voltage components, the ozone formed and the contaminated air.

#### **Experimental Apparatus**

A pictorial representation of the overall experimental setup used by Huang (1996) is shown in Figure 10. The overall setup consisted of a gas handling system, the plasma reactor, an analytical system, and an electrical setup.

### **Electrical System**

Figure 11 depicts the electrical system used. The power source was 110-volt, 60 Hz AC drawn directly from the wall outlet. The applied voltage was stepped-up to 15k-volt using a Jefferson electric luminous transformer. In order to vary the applied secondary voltage to the reactor, a Variac was connected between the wall outlet and the primary side of the transformer. A Simpson, model 210 ammeter was connected before the

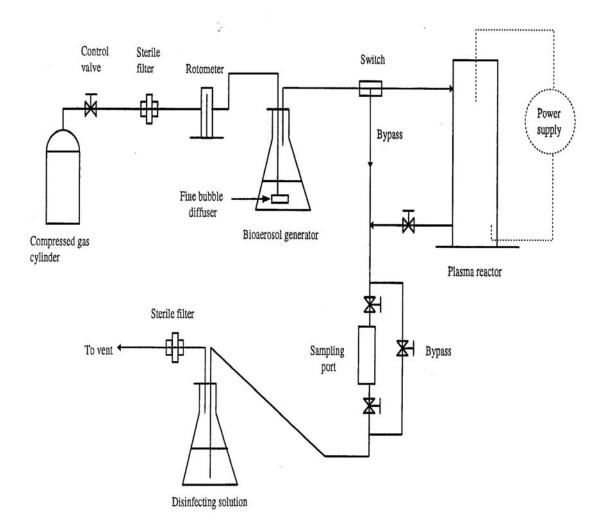


Figure 10 Overall Apparatus Setup

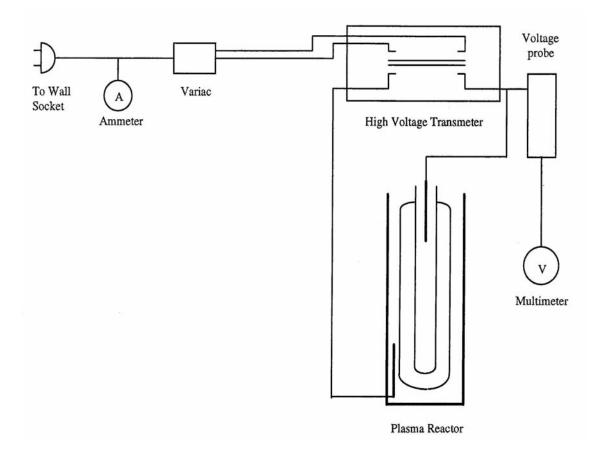


Figure 11 Electrical System

Variac to measure the current on the primary voltage side. The secondary voltage delivered to the reactor by the transformer was measured with the help of a hi-voltage probe, Fluke model 80K-40, with a rated accuracy of  $\pm 5\%$  at 60Hz. To measure the voltage readings, output of the voltage probe was connected to a multimeter (Radio Shack, model No22-166B), with a rated accuracy of  $\pm 1.3\%$  at 60Hz AC.

### **Plumbing System**

The plumbing system starts with a zero-grade compressed gas cylinder (air or nitrogen) fitted with a control valve to regulate the flow. The gas from the cylinder flowed through a sterile filter (Gelman, Acro 50A, 0.45  $\mu$ m filter) to remove any microorganism or dust present in the gas (air/nitrogen). A rotometer was connected after the sterile filter to control and measure the flow rate. Following the flowmeter was the bioaerosol generator which consisted of a fine bubble diffuser placed inside a 1-L Erlenmeyer flask containing the bacteria suspension solution. The bioaerosol laden gas can either be sent to the plasma reactor through the switch or it could be bypassed to the sampling port with the help of the switch. The air from the reactor or the sampling port, is passed through a disinfecting solution (bleach agent) to kill the remaining microorganism and then is passed through a sterile filter and then finally exhausted into the fume hood which vents the air to the atmosphere outside the building.

For the particulate-effect test, a modified plumbing system was used. In addition to the existing flow system, a column containing powdered activated carbon (PAC) (Elf Atochem North America Inc., 3000 mesh) is added. Figure 12 gives a pictorial

representation of the particulate setup used. A compressed air cylinder, fitted with the same sterile filter as mentioned earlier, followed by a rotometer was used to fluidize the carbon in the column. A Y glass connector was utilized to add the particulate flow line to the bioaerosol flow line immediately before the two-way switch. This setup provided a 14 mL mixing zone (approximately  $0.20 \sim 0.35$  seconds of mixing time). The turbulence inside the switch also helped gain additional mixing between bioaerosol and particulates. All the individual units of the gas handling system were connected with a 0.25 in (inner diameter) Tygon tubing.

### **Plasma Reactor**

The reactor (Figure 13) has two concentric glass cylinders inside a plastic outer cylinder. The bioaerosols pass through the space between the inner concentric glass cylinders. The innermost glass cylinder and the volume between the outer glass cylinder and the plastic cylinder were filled with water. High voltage leads from the transformer were immersed in the water contained in the inner glass cylinder and in between the glass and plastic cylinder, thus making water the electrodes for the reactor.

### **Analysis System**

The bioaerosol flow was sent through the bypass or the sampling port by adjusting the three control valves (one before and one after the sampling port and one for the bypass). For sampling, the flow stream was passed through a sterile filter (Micron Separations, Inc., Micronsep-1, 0.45µm) loaded on a pre-autoclaved filter holder (Nalgene, No. 300-4000) for two minutes.

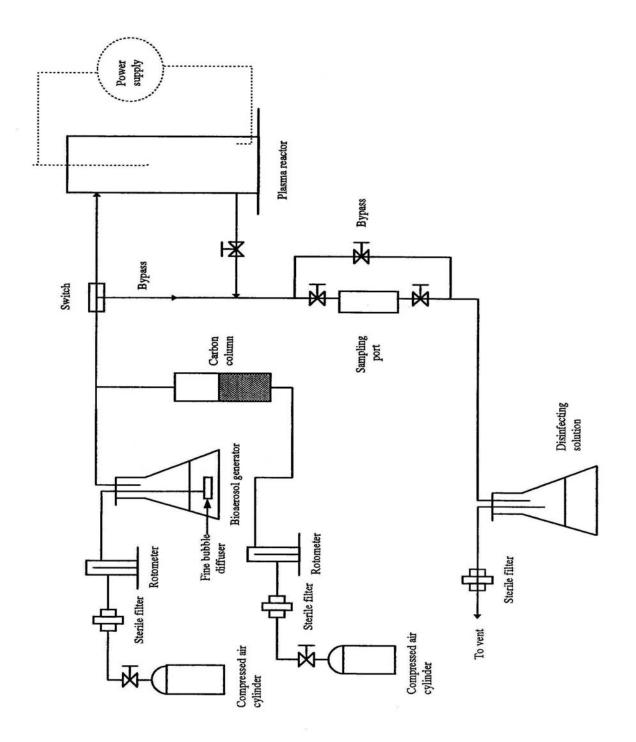


Figure 12 Apparatus set up for Particulate Test

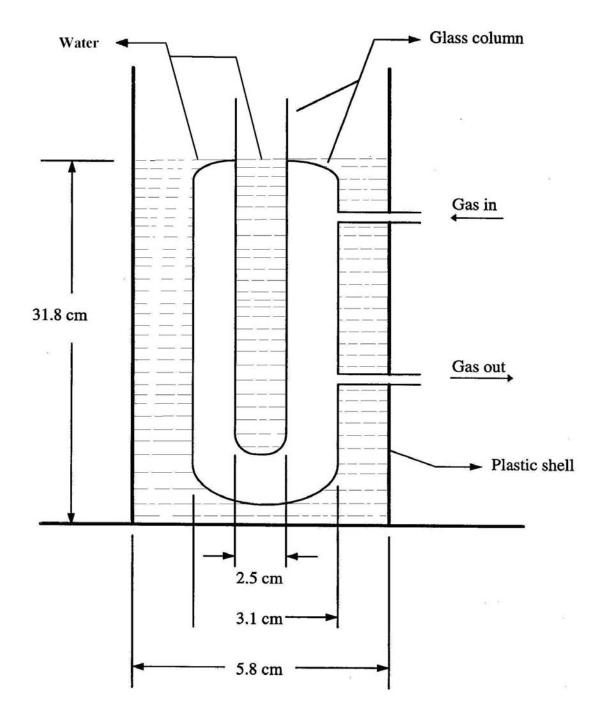


Figure 13 Plasma Reactor

The flow from the plasma reactor or the flow from the sampling filter was passed through a scrubber bottle containing a disinfectant (e.g. bleach agent) to kill any bacteria and then through a  $0.45\mu$ m filter before the bioaerosol flow was vented into the atmosphere through the fume hood.

#### Microbes used and their Characteristics

The microbes used in our test are *E. coli* (gram negative) (American Type Culture Collection 26 or ATCC 26) and *B. subtilis* (gram positive) (ATCC 19659). *E. coli* is generally used in tests because of its clinical significance, *B. subtilis* is a good bioagent stimulant (Kelly-Wintenberg et al., February 1999), and it's a spore forming bacteria, these spores are resistant to heat, radiation and poisonous chemicals (Bailey et al., 1977) and hence was tested in Huang's (1996) experiments. *E. coli* is widely found in intestines of animals – domestic and wild and also in human beings.

#### **Bacterial Solution Preparation**

#### Preparation of E. coli (ATCC26) Solution

The original culture was purchased from ATCC. The culture for the experiments were prepared by adding 2mL of the frozen culture of *E. coli* to 500mL of 8g/L sterilized nutrient broth (Difco). To provide oxygen for growth the cultures were incubated at room temperature (19°C) on an automatic shaker and were harvested 24 hours later. Sterile dilute water was added to dilute this bacterial solution so that a readable number of colony forming units (CFU) could be obtained on a 47-mm filter (Micron Separations, Inc., Micronsep-1, 0.45 $\mu$ m) during a trial run. This bacterial solution, after appropriate dilution, was stored in a refrigerator at a constant temperature of 4°C for two weeks before use. Three bacterial such solutions were prepared and were grown over the two and a half months duration of the experiments.

### Preparation of B. subtilis (ATCC 19659) Solution

The original culture was brought from ATCC. To prepare a *B. subtilis* solution, one 2mL aliquot of frozen culture of *B. subtilis* was added to 500 mL of 30mg/L sterilized tryptose broth and was incubated at 37°C for 24 hours. In order to achieve sporulation, while harvesting, 10 mL of the solution was transferred to tryptose nutrient agar (Difco) plates (Fisher, 100X15mm Petri plates) and incubated at 37°C for 7 days. The harvested spores were then suspended in 10 mL of distilled water by rubbing the agar surface with a sterile rubber policeman. As in *E. coli* preparation, the bacterial suspension was diluted with sterile dilution water until a countable number of colonies could be obtained on a 47mm filter. This solution was then heated in a water bath at 80°C for 10 minutes to kill the remaining vegetative cells. The culture suspension was stored at 4°C in a refrigerator for three days before use. Only one *B. subtilis* solution was prepared during the entire course of the experiments.

### **Experimental Design**

The variables used in the experiments were the test bacteria, power input, carrier gas and residence time. Two different microorganisms (*E. coli* and *B. subtilis*), and three different power inputs (27, 38, 68 Watts corresponding to 7500, 9500, 12,500 Volts) were used. The residence time, in seconds, used were 0.4 s and 0.7 s. The carrier gases used were air and nitrogen. Two additional tests were conducted during the particulate-

contaminated air plasma experiments to measure the particulate concentration in the flow. Table 1 shows the matrix used for air and nitrogen plasma.

Residence time Seconds	Flow rate L/min	Secondary voltage Volts (V)	Power input Watts (W)	Bacteria used
0.4	4.5	7,500	27	E. coli
0.4	4.5	9,500	38	E. coli
0.4	4.5	12,500	68	E. coli
0.7	2.5	7,500	27	B. subtilis
0.7	2.5	9,500	38	B. subtilis
0.7	2.5	12,500	68	B. subtilis

Table 1. Matrix of variables used for air and nitrogen as carrier gas

Before starting the carrier gas flow (air or nitrogen), all the switches, valves and tubing connections were checked for leaks. The carrier gas was passed through the Erlenmeyer flask containing a solution of the test microorganism for 10 minutes to generate a stabilized bioaerosol concentration in the carrier gas. This initial flow was bypassed and did not flow through the plasma reactor. After the 10-minute period the flow was directed to pass through the sampling port for 2 minutes. This allows the determination of the number of bacteria dispersed in the air phase.

After sampling, the flow was switched to the reactor with the help of the two-way switch and the reactor was turned on at the power supply at the required setting (as shown in Table 1). In order to obtain a steady bioaerosol concentration in the carrier gas no sample was collected during the first five minutes of operation of the plasma reactor. After the first five minutes, three effluent samples were collected at five minute intervals between each sample. These three effluent samples helped evaluate the destruction of bacteria under the set operating conditions.

After the collection of all the effluent samples the power to the reactor was switched off and the flow was continued through the reactor for another 7 minutes through the reactor and another sample was collected after the 7<sup>th</sup> minute. This sample was used to verify the bacteria in the influent stream before the reactor was turned on. Finally one more sample was collected after bypassing the flow around the reactor for 5 minutes. For each sampling event a new sterilized sampling filter was used.

In case of the particulate testing, after the first two-minute sampling for bioaerosols, another sample was collected at the 5<sup>th</sup> minute to measure the particulate concentration in the carrier gas before the flow was introduced into the reactor. And likewise, at the end of the run, another sample was collected five minutes after the 7<sup>th</sup> minute sampling to verify the particulate concentration inside the reactor when it was off. In addition to these samples one final sample was taken after by-passing the flow around the reactor for five minutes. This sample helped confirm the bioaerosol concentration at the end of the run.

Other measurement done during the course of the experiments included: humidity and temperature measurement, ozone measurement and a negative control.

### **Humidity and Temperature Measurement**

Relative humidity (RH) was experimentally determined by Huang using the method suggested by Miller (1966). Calcium sulfate was used in this test to measure the humidity at a flow rate of 2.5 L/min. The flow was directed through the sampling port of the analysis system which was replaced with a column containing 152.152 grams of anhydrous calcium sulfate. A gradual change in the color of calcium sulfate, from blue to pink, was noted due to the absorption of water molecules on calcium sulfate. The test was carried out until two thirds of the calcium sulfate's color changed to pink, and the time required was 60 minutes. The flow was timed so that the weight of water added to the calcium sulfate could be calculated. The difference in weight between the wet and dry calcium sulfate gives the weight of water collected. The relative humidity (RH) can be calculated using the formula (Huang, 1996):

$$RH = (W_{wf}/W_{af}) X 100\%$$
(7)

Where,

 $W_{wf}$  = weight of water collected from the flow in grams

 $W_{af}$  weight of air in the flow in grams.

Temperature in the flow was measured using a thermometer/hygrometer (Universal Enterprises, Inc., Model DTH1) connected to the outlet of the reactor.

### **Ozone Measurement**

Ozone is produced due to neutral particle conversion in a discharge zone (Efremov et al., February 2000). The corona discharge method is commercially used to produce ozone.

Ozone production efficiency depends on factors like gap width, gas pressure, properties of dielectric and metal electrodes, power supply and moisture (Khurana, 2003).

The Iodometric method was used to determine the ozone concentration (143 A. Iodometric method, Standard Methods for the Examination of Water and Wastewater, 13<sup>th</sup> edition, American Public Health Association, NY). Two 500 mL glass washing bottles were each filled with 400 mL of 0.05N KI solution and these bottles were connected in series by a 0.25 in (ID) tygon tube and were attached to the sampling port. The carrier gas was sent through the plasma reactor and from the reactor to the sampling port.

The gas flow was started at the desired flow rate and the reactor was turned on at the desired voltage and simultaneously a stop watch was started. When the KI solution in the second bottle turned faint yellow the flow was bypassed and the time on the stop watch was noted. The KI solutions from both the bottles were transferred to a one-liter beaker and 10mL of 20% H<sub>2</sub>SO<sub>4</sub> was added to it. Now this solution was titrated against 0.05 N Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub>, taken in a burette, until the yellow color disappeared. To this solution (colorless) 5mL of starch indicator was added to give it a blue color. This solution was again titrated against Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> until the blue color disappeared. The volume of Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> required to render the blue solution colorless was noted. This procedure was repeated for different secondary voltages. The ozone concentration was calculated using the formula: O<sub>3</sub> (mg/L) = (mL of titrant x 0.05N x 24000)/V<sub>g</sub> (8)

 $V_g$  = Amount of gas passed through the gas washing bottles in mL.

## **Power Input**

In order to determine the power input into the system, a set of runs were done with dry air at an airflow rate of 2.5L/min. The results are displayed in Table 2. From Figure 14, it can be inferred that with an increase in applied secondary voltage the power input increases exponentially. The primary voltage that was input to the system was 110 volts and the power input was determined by multiplying the primary voltage with the primary current.

Primary Current	Power Input (Primary voltage x Primary current)	Secondary Voltage
Amps	Watts	kVolts
0.62	68.2	13.0
0.54	59.4	12.3
0.42	46.2	10.9
0.32	35.2	9.6
0.25	27.5	8.1
0.22	24.2	7.4
0.19	20.9	6.6

 Table 2. Electrical measurements for 110V Primary Voltage

## **Minimum Voltage**

It was found out that a minimum voltage of 7kV was required to maintain an uniform glow plasma at room temperature and one atmosphere pressure. This value helped in establishing the secondary voltages of 7,500 V, 9,500 V and 12,500 V used in this work.

With the increase in voltage the brightness of the glow increased due to higher ionization and excitation.

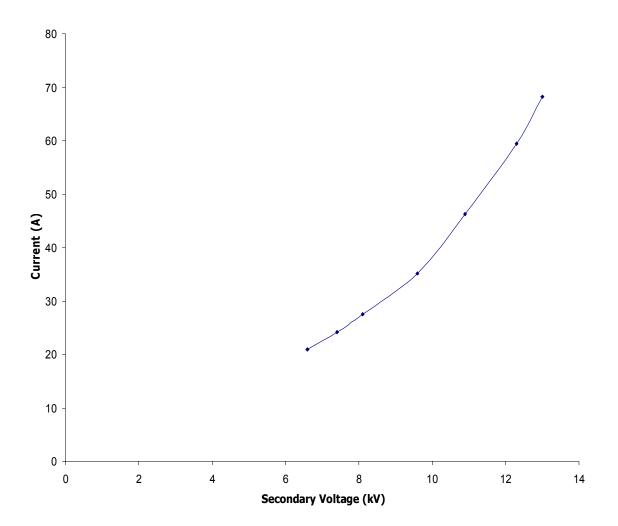


Figure 14. Secondary voltage Vs Current – Huang (1996)

# **Secondary Current**

Secondary current was calculated using the current error % formula suggested by National Physical Laboratory (NPL) (2005).

Current error (%) = 100 (K<sub>N</sub> I<sub>S</sub> - I<sub>P</sub>) / I<sub>P</sub> (9)

Where,

 $K_N$  = transformer ratio  $I_S$  = actual secondary current

 $I_P$  = actual primary current

Since secondary current could not be measured directly, current error percentage was assumed at 25%, 50%, 75%, and 100% and the respective secondary currents were calculated using the above stated formula.

### **Secondary Power**

The secondary power  $(P_S)$  was calculated by multiplying the secondary current with the secondary voltage (secondary current \* secondary voltage) that is obtained using the high voltage probe. Since secondary current was calculated assuming error percentages the secondary power and energy density values were also calculated using the assumed error percentage values.

### **Energy Density**

Energy density is usually represented in Joules/Liter. Energy density value helps in estimation of power requirement for the given destruction efficiency and gas flow rate (Agnihotri et al, 2004). The formula used by Huang (1996) was modified and was used to obtain energy density in J/L.

Energy Density  $(E_d) = (P_S \times R.T.)/V_R$  (10) Where,

 $E_D$  = Energy density in Joules/Liter

 $P_S$  = Secondary power in kV

R.T. = Residence Time in seconds

 $V_R$  = Volume of reactor in L

#### **Reynolds Number**

Reynolds number was calculated (Appendix C) using the following formula (McCabe et

al, 1993):

Reynolds Number  $(N_{Re}) = (D \times V \times \rho)/(\mu)$  (11)

Where,

 $N_{Re}$  = Reynolds number (dimensionless unit)

D = diameter, cm

V = velocity of flow, cm/s

 $\rho$  = density of air, Kg/cm<sup>3</sup>

 $\mu$  = dynamic viscosity, Kg/cm-s

### **Cost of Operation**

The cost of operating the DBD plasma reactor was calculated assuming the cost of power to be at \$0.10 per kilowatt hour.

#### **Negative Control**

A negative control was implemented every time before starting any experiment to assure sterility of the system. All the connecting (Tygon) tubing was sterilized by passing isopropyl alcohol through it. The reactor was run for 5 minutes in order to sterilize it. After air-drying the treated Tygon tubing for 24-hours, the system was reassembled and the setup was sampled with the help of a sample filter. This filter was incubated at 35°C on a nutrient broth and examined after 24 and 48 hours for cell growth.

### **Chapter IV**

### **Results and Discussion**

Significant destruction of *E. coli* and *B. subtilis* was obtained for the various test parameters considered. An overall destruction efficiency of 100% was obtained with air as the carrier gas. Apart from destruction efficiency energy density and error percentage also have been calculated.

### **Ozone Production**

The ozone produced at various conditions is listed in Table 3. When air was used as carrier gas the ozone concentration ranged from 0.8 to 0.05mg/L. Ozone tests were done with nitrogen as carrier gas to examine the production of ozone. Iodometric tests revealed no ozone production when nitrogen was used as the carrier gas. This is due to the absence of oxygen molecules in nitrogen gas. It could also be concluded that with increase in applied power, ozone concentration increased. A similar trend was noticed with an increase in residence time. Both the trends are in accordance with observations noted by Horvath et al (1985).

Residence Time = 0.4 s		Res	Residence Time = $0.7 \text{ s}$		
Power Input	Carrier gas	Ozone Concentration	Power Input	Carrier gas	Ozone Concentration
Watts		mg/L	Watts		mg/L
68	Air	0.30	68	Air	0.80
38	Air	0.15	38	Air	0.34
27	Air	0.05	27	Air	0.13
68	$N_2$	0.0	68	$N_2$	0.0
38	$N_2$	NC	38	$N_2$	NC
27	N <sub>2</sub>	NC	27	N <sub>2</sub>	NC

Table 3. Ozone Generation at Various Conditions

NC - Test was not conducted for this condition

### **Relative Humidity**

Relative humidity was determined according to the procedure cited and for this setup it was 71.2% at a temperature of 19 °C. Detailed calculations can be found in Appendix B.

### **Residence** Time

The reactor has a volume of 28 mL (annulus). The flowrates used were 2.5mL/min and 4.5mL/min and hence the residence times were 0.7 and 0.4 seconds respectively.

#### **Bacterial Concentration in Air Stream**

The bacteria were collected on the sample filter and were grown on specific growth media and were counted, but a limitation with this method is that too many colonies on the media can result in crowding and colony overlaps, ultimately resulting in counting errors. Huang (1996) reported that the maximum number of bacteria that could be counted without any errors was 700 CFUs and any number higher than this was listed as Too Many CFUs To Count (TMTC).

The bacteria in the career gas were calculated by Huang (1996) using the formula:

$$C = N/(Q x t)$$

(12)

Where,

 $C = bacterial content in gas, CFUs/ft^3$ 

N = number of CFUs

- $Q = gas flow rate, ft^3/min$
- t = collection period, minutes

C has been representd in CFUs/ft<sup>3</sup> to facilitate easy comparison with the results reported by other authors. For a flow of 2.5L/min the influent bacterial concentration was in the range of 980 to 3200 CFU/ft<sup>3</sup> and for a flow of 4.5L/min the concentration ranged from 525 to 1800 CFU/ft<sup>3</sup>. For comparison Table 4 lists the ambient airborne bacteria concentrations reported by various other authors. Bed making in a civilian hospital closely matches the bacterial concentration range used in the experiment.

### **Reynolds Number**

The Reynolds number for the two flow rates was calculated and they were 1386.7 and 770.4 for 4.5 and 2.5 L/min respectively, proving that the flow through the reactor is laminar (McCabe et al, 1993).

Author	Airborne bacteria concentration CFU/ft <sup>3</sup>	Location
Tsai et al (2002)	8	Indoor concentration for 100
		buildings selected in random over
		the three seasons
Beggs (2003)	174	Vigorous bed making inside a patient room in a hospital
Bourdillon et al (1948)	2000	Bed making in a civilian hospital

 Table 4. Airborne bacteria Concentration Reported in the Lliterature

### Results

The bacterial concentration has been represented in CFUs in the following section. Appendix D lists the data for all the destruction runs carried out by Huang (1996) with bacterial concentration converted to CFUs/L so that results for various flow rates can be compared on a common basis.

### **Destruction with Air**

The entire data set for experiments with air as carrier gas is listed in Appendix D (Tables D1 –D12). A summary of the tests is given in Table 5. The results proved that neither *B*. *subtilis* nor *E. coli* formed a viable colony after passing through the plasma reactor. The overall destruction efficiency for the two different test bacteria at 6 different operating conditions was 100%. Assuming one bacterial breakthrough the overall efficiency was higher than 99.6%. Graphs were plotted with three divisions, with each division representing: plasma off, plasma on, plasma off, respectively as viewed from left to right. The bacteria concentration goes down to zero during the times the plasma is on and then gets back to the initial value or a value higher or lower than the initial value. Figures 15 – 18 represent graphically the destruction of the two different bacteria for the highest power, longest residence time and lowest power and shortest residence time so that the effect of these parameters on the destruction can be studied.

### **Destruction with Nitrogen**

Part of the destruction achieved could be achieved by ozone that acts as a germicidal agent and is produced in air plasma in addition to the destruction. Therefore the

destruction is due to both electrical action and ozone. Tests were conducted with nitrogen gas instead of air to check the effectiveness of the electrical destruction of bacteria alone, as no ozone is produced in nitrogen due to lack of oxygen. It can also be understood from Table 3 that no ozone is formed in nitrogen plasma. Complete set of experimental data can be found in Appendix D (Tables D13 – D26). The tests were conducted nitrogen as carrier gas. As with the air plasma, the bacteria were tested under the six different operating conditions. B. subtilis occurred four out of six times in the nitrogen plasma effluent while no breakthrough occurred with air plasma. Bacterial breakthrough for *B. subtilis* occurred at the highest and lowest power inputs (68 and 27 watts) and also for the longest and shortest residence times (0.7 sec and 0.4 sec)suggesting that power input and residence time had little effect on the destruction efficiency of B. subtilis in nitrogen plasma. B. subtilis breakthrough in nitrogen plasma implies that ozone generated in the air plasma had a role in the destruction of B. subtilis. The effect of ozone on *E. coli* could not be confirmed, as no breakthrough of *E.* coli was observed for the six test conditions using nitrogen as the carrier gas. E. coli is less resistive to environmental changes compared to spores and hence might have been destroyed by the plasma. Overall destruction efficiency was higher than 99.9 % for both the bacteria, using nitrogen plasma.

Figures 19-22 illustrate the destruction in the nitrogen plasma for the highest power, longest residence time and lowest power and shortest residence time. Table 6 gives a summary of the results for the nitrogen plasma.

Test No	Power Input	Residence Time (RT)	Bacteria	No. of bacteria challenged	No. of bacteria breakthrough
	Watt	Seconds		CFU	CFU
B1	68	0.4	B. subtilis	278	0
B2	68	0.7	B. subtilis	394	0
В3	38	0.4	B. subtilis	216	0
B4	38	0.7	B. subtilis	246	0
B5	27	0.4	B. subtilis	216	0
B6	27	0.7	B. subtilis	254	0
E1	68	0.4	E. coli	311	0
E2	68	0.7	E. coli	176	0
E3	38	0.4	E. coli	541	0
E4	38	0.7	E. coli	217	0
E5	27	0.4	E. coli	167	0
E6	27	0.7	E. coli	226	0

Table 5. Summary of Destruction Results for DBD Air Plasma

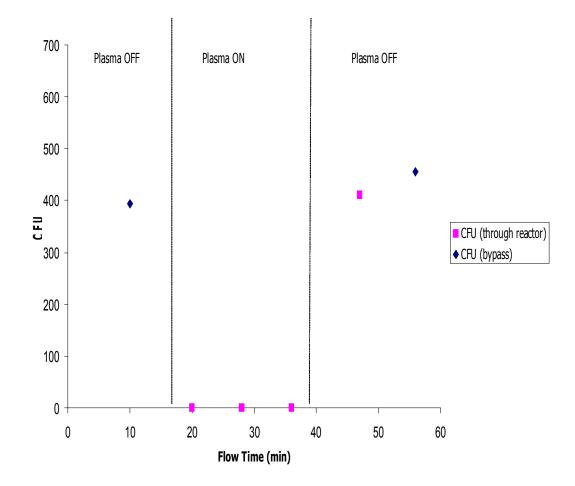


Figure 15. Destruction plot for *B. subtilis* at 68 watt Power, 0.7 sec RT in Air Plasma

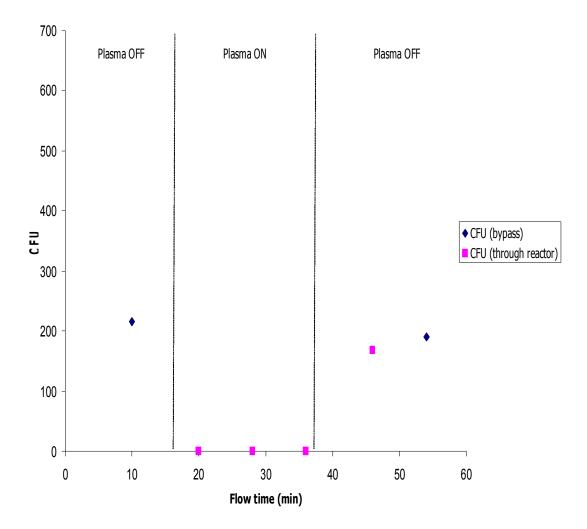


Figure 16. Destruction Plot for *B. subtilis* at 27 watt Power, 0.4 sec RT in Air Plasma

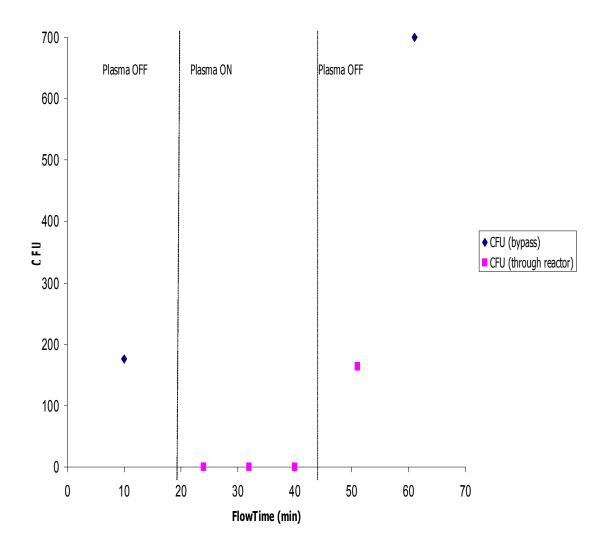


Figure 17. Destruction Plot for E. coli at 68 watt Power, 0.7 sec RT in Air Plasma

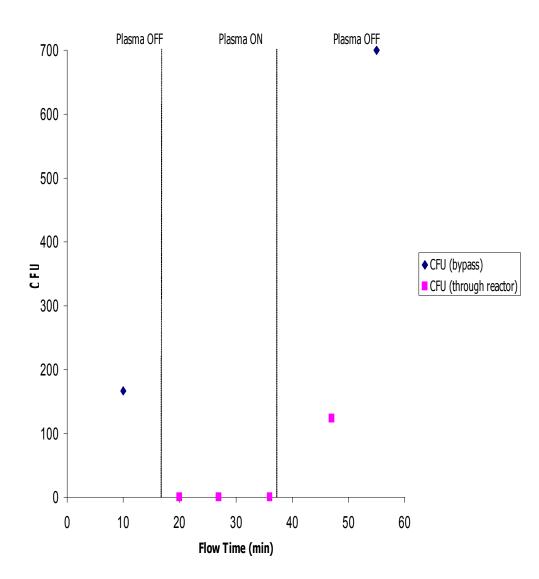


Figure 18. Destruction plot for *E. coli* at 27 watt, 0.4 sec RT in Air Plasma

Test No.	Power Input	Residence Time (RT)	Bacteria	No of bacteria challenged	No of bacteria Breakthrough*
	Watt	Seconds		CFU	CFU
B7	68	0.4	B. subtilis	175	(0,0,1)
B8	68	0.7	B. subtilis	291	(0,1,1)
B9	38	0.4	B. subtilis	180	(1,1,1)
B10	38	0.7	B. subtilis	277	0
B11	27	0.4	B. subtilis	175	0
B12	27	0.7	B. subtilis	292	(1,0,0)
E7	68	0.4	E. coli	228	0
E8	68	0.7	E. coli	382	0
E9	38	0.4	E. coli	235	0
E10	38	0.7	E. coli	567	0
E11	27	0.4	E. coli	375	0
E12	27	0.7	E. coli	478	0

Table 6. Summary of destruction results for nitrogen plasma

\* - represents the number of bacteria breakthrough from the second, third and fourth samples, the first sample being the influent sample.

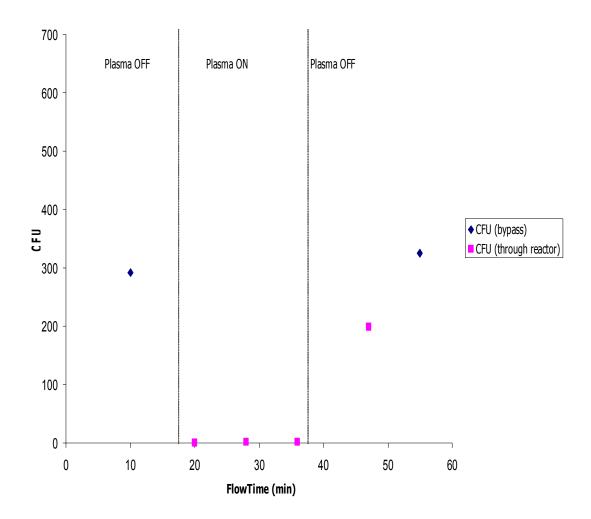


Figure 19. Destruction Plot for *B. subtilis* at 68 watt Power, 0.7 sec RT in Nitrogen Plasma

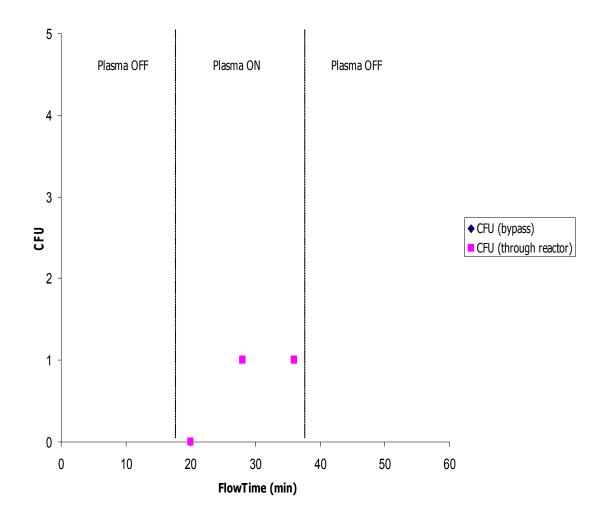


Figure 20. Destruction plot for *B. subtilis* (Figure 20) with magnified axis to show breakthrough

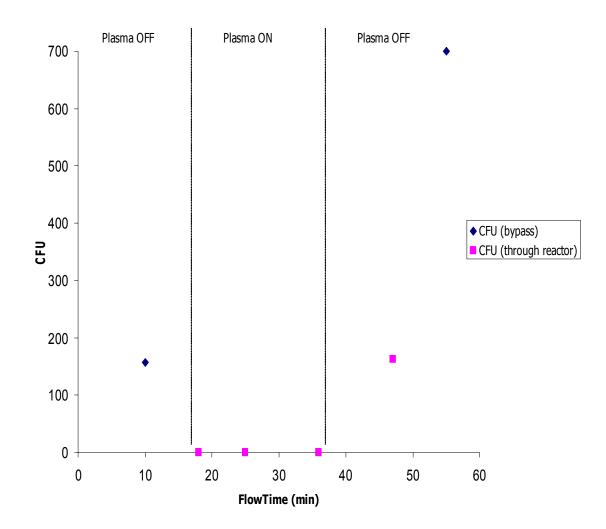


Figure 21. Destruction plot for *B. subtilis* at 27 watt, 0.4 sec RT in Nitrogen Plasma

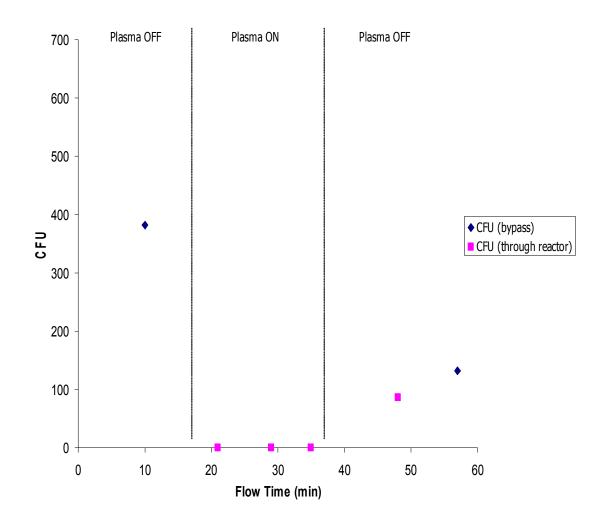


Figure 22. Destruction plot for *E. coli* at 68 watt Power, 0.7 sec RT in Nitrogen Plasma

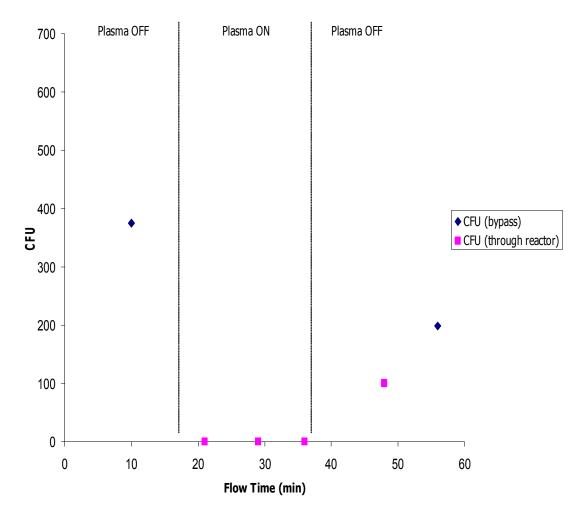


Figure 23. Destruction plot for *E. coli* at 27 watt Power, 0.4 sec RT in Nitrogen Plasma

#### **Bacterial Destruction in Particulate-Contaminated Air Plasma**

Air containing dispersed particulate matter was simulated by introducing carbon particles into the air stream. This test was done only air as carrier gas. The entire set of data can be found in Appendix D (Tables D27 –D38). A summary of the destruction results for the particulate test is listed in Table 8. Overall the particulate concentration in the reactor varied by a minimum of 0% and a maximum of 33%.

The initial and final weight of carbon particulates in the air stream was measured and the particulate concentration was calculated according to the following formula: Particulate Concentration  $(g/m^3) = (W_c)/(Q \times t)$  (11) Where,

 $W_c$  = weight of carbon collected, grams

 $Q = air flow rate, m^3/min$ 

t = sampling time, minutes

The particulate concentration used during this series of tests ranged from 27,000  $\mu$ g/m<sup>3</sup> to 80,000  $\mu$ g/m<sup>3</sup>, which is much higher than EPA's standards of 150  $\mu$ g/m<sup>3</sup> (averaged over 24 hours) for particulate matter in ambient air. Irrespective of the test parameters no bacterial breakthrough was observed. The overall efficiency was higher than 99.5% for the six variables (calculated in a conservative manner – assuming one bacterial breakthrough). The effect of power, residence time on bacterial destruction could not be estimated. This led to the conclusion that the presence of particulates in the air plasma does not deter the plasma's efficiency.

Test No.	Power Input	Residence Time	Bacteria	No. of bacteria challenged	No. of bacteria Breakthrough
	Watt	Seconds		CFU	CFU
	60				
B15	68	0.4	B. subtilis	214	0
B16	68	0.7	B. subtilis	189	0
B17	38	0.4	B. subtilis	232	0
B18	38	0.7	B. subtilis	187	0
B19	27	0.4	B. subtilis	267	0
B20	27	0.7	B. subtilis	203	0
E13	68	0.4	E. coli	502	0
E14	68	0.7	E. coli	298	0
E15	38	0.4	E. coli	353	0
E16	38	0.7	E. coli	287	0
E17	27	0.4	E. coli	567	0
E18	27	0.7	E. coli	87	0
	I	I		l	I

Table 7. Summary of destruction results with particulates

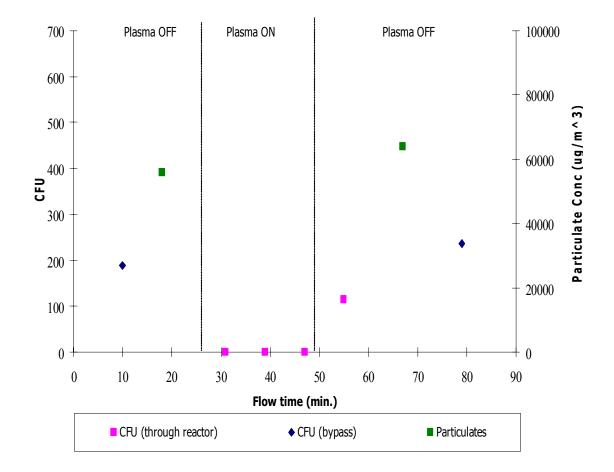


Figure 24. Destruction of *B. subtilis* at 68 watt Power, 0.7 sec RT in Air Plasma with Particulates

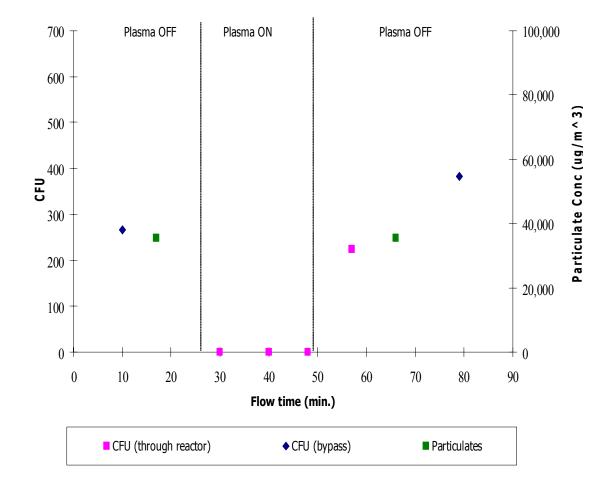


Figure 25. Destruction plot of *B. subtilis* at 27 watt Power, 0.4 sec RT in Air Plasma with Particulates

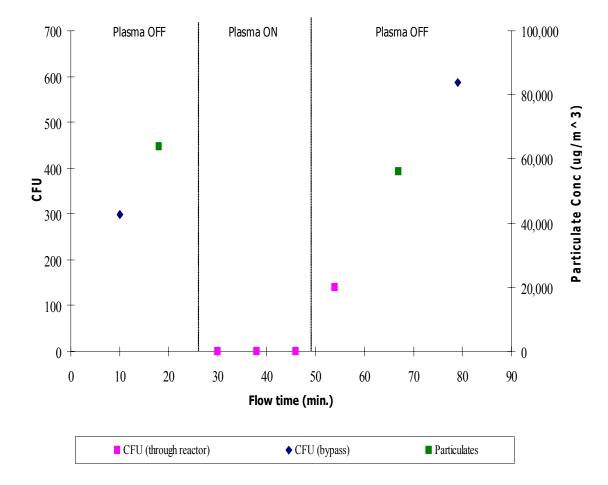


Figure 26. Destruction plot for *E. coli* at 68 watt Power, 0.7 sec RT in Air Plasma with Particulates

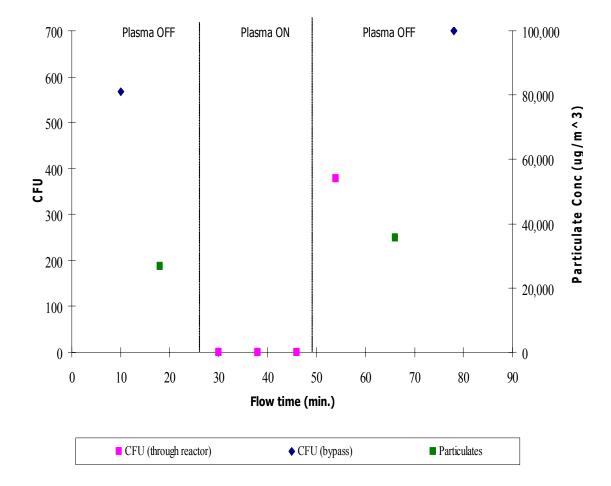


Figure 27. Destruction plot for *E. coli* at 27 watt Power, 0.4 sec RT in Air Plasma with Particulates

## **Energy Density**

Assuming error percentages of 25%, 50%, 75%, and 100% in secondary current calculation a set of energy density values were obtained. Tables 8 –11 list the energy density values. It was observed that energy density always increased with increase in secondary voltage. It was also evident from the results that energy density was always higher for longer residence times. Calculations for secondary power and energy density can be found in Appendix E.

Secondary Power	Reactor Volume	Energy Density at Residence Time			
		0.4 sec 0.7 sec			
watt	L	J/L	J/L		
61.8	0.028	882.1	1543.8		
26.8	0.028	382.2	668.8		
18.8	0.028	267.9	468.8		

 Table 8. Energy Density - assuming 25% error

Table 9. Energy Density - assuming 50% error

Secondary Power	Reactor Volume	Energy Density at Residence Time	
		0.4 sec 0.7 sec	
watt	L	J/L	J/L
74.1	0.028	1058.6	1852.5
32.1	0.028	458.6	802.6
22.5	0.028	321.4	562.5

	• 0				
Secondary Power	Reactor Volume	0,	Energy Density at Residence Time		
2 · · · · · · · · · · · · · · · · · · ·		0.4 sec	0.7 sec		
watt	L	J/L	J/L		
86.5	0.028	1235	2161.3		
37.5	0.028	535.1	936.3		
26.3	0.028	375	656.3		

Table 10. Energy Density - assuming 75% error

## Table 11. Energy Density - assuming 100% error

Secondary Power	Reactor Volume		Density at ce Time		
		0.4 sec 0.7 sec			
watt	L	J/L	J/L		
98.8	0.028	1411.4	2470		
42.8	0.028	611.5	1070		
30	0.028	428.6	750		

#### **Comparison with previous results**

The results obtained by Huang (1996) were compared against Schroeder's (1996) work on destruction of *E. coli* and *B. subtilis* using the same plasma reactor. Overall there were fewer breakthroughs in Huang's work (1996) when compared to Schroeder's (1996) work for similar settings which implies that Huang (1996) had better destruction than Schroeder (1996). It was also noted that the bacterial concentration was lower in Schroeder's research. For example, for a flow of 4.5 L/min at 9,500 v for the destruction of *B. subtilis*, Schroeder's (1996) initial concentration in CFU/L (before destruction) was 6.57 whereas Huang's initial concentration was 24. Table 9 compares the two sets of results for 9,500v input voltage at a flow rate of 4.5 L/min for *B. subtilis*.

	Huang	g (1996)			Schroe	der (1996)	
time (min)	Plasma status (on/off)	Flow path	Bacteria (CFU/L)	time (min)	Plasma status (on/off)	Flow path	Bacteria (CFU/L)
0	off	bypass	24	0	off	bypass	7
9	on	reactor	0	9	on	reactor	0
17	on	reactor	0	16	on	reactor	0.04
25	on	reactor	0	24	on	reactor	0.04
35	off	reactor	19	31	off	reactor	3
43	off	bypass	21	40	off	bypass	TMTC*

Table 12. Comparison of Data with previous research	Table 12.	Comparison	of Data with	previous	research
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\*TMTC – Too Many colony forming units To Count.

Schroeder (1996) tested the ozone production on the same reactor and under same conditions with air plasma (Table 13). The ozone produced from Huang's reactor (1996) differed from Schroeder's (1996) only by 0.5%.

Residence Time = 0.4 s			Residence Time = $0.7$ s		
Power Input	Carrier gas	Ozone Concentration	Power Input	Carrier gas	Ozone Concentration
Watts		mg/L	Watts		mg/L
60	Air	0.296	60	Air	0.796
34.8	Air	0.146	34.8	Air	0.340
30	Air	0.053	30	Air	0.129

Table 13. Ozone Generation – Schroeder (1996)

Huang's (1996) electrical data was checked against Schroeder's (1996). Schroeder used the same reactor and the same electrical setup but the primary voltage used by Schroeder was 120 volts. On comparing the two electrical data sets (Table 2 and Table 14) it can be seen that there is a negligible difference.

Primary Current	Power Input (Primary voltage x Primary current)	Secondary Voltage
Amps	Watts	kVolts
0.50	60	12.5
0.41	49.2	11.5
0.34	40.8	10.5
0.29	34.8	9.5
0.25	30	7.5

 Table 14. Electrical Measurements for 120v primary (Schroeder, 1996)

#### **Error Percentages**

Error percentages were calculated for all the data sets obtained from Huang's (1996) and Schroeder's (1996) work. It was seen that, in both the works, the error percentage was higher for *E. coli*. Table 15 compares Huang's (1996) average error percentage for runs B1 - B6 and E1 - E6. In case of *B. subtilis* the difference between the initial concentration and the concentration when the reactor is off during the run was 8.8% but in case of *E. coli* it was 32.1%. The error percentage for bypass was negative as the bacterial concentration when the flow was bypassed was higher than the initial concentration/initial reading. Appendix F contains the error percentage for all the data including error percentage for Huang's verification data in Appendix G.

Destania	Derrice	Comin	Averag	ge Error %
Bacteria	Run series	Carrier Gas	Reactor-off	Bypass
B. subtilis	B1 – B6	Air	8.8	-9.3
E. coli	E1-E6	Air	32.1	-300

Table 15. Error % comparison between *B. subtilis* and *E. coli* 

#### Chapter V

#### **Conclusions and Recommendations**

From the work done it can be safely concluded that DBD technology can be utilized to achieve an overall destruction efficiency of 100% for bacterial inlet concentrations 3.5 times higher than the maximum concentration of bacteria generated due to normal morning activity in a service hospital (Bourdillon et al., 1948). Further study on the effect of voltage, frequency, relative humidity and residence time must be done to optimize the parameters.

#### Conclusions

- The power input into the system was 27, 38 and 68 watt and the corresponding voltages were 7500, 9500 and 12,500 volts which demonstrates that power input increases with increase in voltage. The cost of operating the alternating current plasma reactor for the above mentioned power wattages were \$ 0.0027, \$ 0.0038, \$0.0068 per hour.
- 2) No conclusions could be made on the effect of power and residence time on the destruction efficiency as there was no bacterial breakthrough for all the tests excluding *B. subtilis* in nitrogen plasma. Even though there were breakthroughs in nitrogen plasma no conclusion could be made as the breakthroughs occurred at the highest power and highest residence time and lowest power and lowest residence time.
- The overall efficiency for DBD air plasma, at the various operating conditions was higher than 99.6%

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- 4) Concentration of ozone generated from the air plasma was in the range of 0.05 mg/L to 0.8 mg/L. Ozone generated from the DBD air plasma increased with increase in voltage. The same trend was noticed for increase in residence time. The ozone concentration obtained by Huang (1996) differs from Schroeder's (1996) work only by 0.5%
- 5) Ozone generated during the operation of the plasma reactor also had a role in the destruction mechanism of *B. subtilis*.
- 6) Power and residence time had no effect on the destruction of *B. subtilis* in the nitrogen plasma. *E. coli* breakthrough did not occur in the nitrogen plasma.
- The nitrogen plasma, for the different operating conditions, had a destruction efficiency higher than 99.9%
- 8) Presence of particulate matter in the carrier stream did not affect the operation of the plasma reactor. Efficiency higher than 99.5 % was obtained in DBD air plasma with particulate matter.
- 9) Huang's (1996) work had a higher efficiency compared to Schroeder's (1996) on the same reactor. Also Huang (1996) reported lesser bacterial breakthroughs compared to Schroeder (1996) for the same reactor and similar operating conditions.
- 10) The error percentages for all of Huang's (1996) were done. *E. coli* had the highest error percentage and the same trend was noticed in Schroeder's (1996) work.

11) The energy density, assuming 100% error in secondary current calculation, is in the range of 429 - 1411 J/L for a residence time of 0.4 s and 750 - 2470 J/L for a residence time of 0.7 s

#### Recommendations

- The number of countable CFUs was limited to 700 as CFUs greater than 700 crowded the in-line membrane filter, thereby making it difficult to count. Use of impingers, which have a much wider range (Jensen et al., 1998), is recommended.
- 2) A wider range of power and residence times should be used to arrive at optimal values for the destruction of *B. subtilis* and *E. coli*. Also, the effect of change in frequency on the destruction efficiency for *B. subtilis* and *E. coli* should be studied. Electrical data necessary to calculate energy density should be measured directly for all the operating conditions used in future tests. Energy density helps in scale-up calculations.
- 3) Collection of samples should start once the reactor is turned on and consecutive samples must be collected in a short time gap (in the order of seconds) to better understand the destruction mechanism of the plasma reactor and to establish a Time destruction relation. Sample flow time can be reduced from 2 min to a few seconds. This permits multiple sampling within a minute.
- 4) Omission of nonculturable bacteria leads to underestimation of the bioaerosol concentration. Future tests should analyze the sample for both culturable and nonculturable bacteria to calculate the exact bioaerosol concentration.

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# APPENDIX A

Reactions in a Plasma Reactor

## The Chemistry of Silent Discharge

The main plasma reactions are electron/molecular reactions, atomic/molecular reactions, decomposition, and synthesis. In the ensuing reactions A, B represent atoms,  $A_2$ ,  $B_2$  represent molecules and e is used for electrons, M stands for temporary collision partner, ions have a + or – superscript, and the excited species are superscripted with an asterisk. All the equations listed below are from the works of Eliasson and Kogelschatz, 1991b and they have been represented below without any modifications.

#### **Electron/molecule reactions**

Excitation:

$$e + A_2 \rightarrow A_2^* + e$$

Dissociation:

$$e + A_2 \rightarrow 2A + e$$

Attachment:

$$e + A_2 \rightarrow A_2$$

Dissociative Attachment:

$$e + A_2 \rightarrow A^- + A^-$$

Ionization:

 $e + A_2 \rightarrow A_2^+ + 2e$ 

Dissociative Ionization:

 $e + A_2 \rightarrow A^+ + A + e$ 

Recombination:

$$e + A_2^+ \rightarrow A_2$$

Detachment:

 $e + A_2^- \rightarrow A_2 + 2e$ 

## Atomic /molecular reactions

Penning Dissociation:

 $M^* + A_2 \rightarrow 2A + M$ 

Penning Ionization:

 $M^* + A_2 \rightarrow A_2^+ + M + e$ 

Charge Transfer:

 $A^{\pm} + B \longrightarrow B^{\pm} + A$ 

Ion Recombination:

 $A^- + B^+ \rightarrow AB$ 

Neutral Recombination:

 $A+B+M \ \rightarrow \ AB+M$ 

## Decomposition

Electronic:

 $e + AB \rightarrow A + B + e$ 

Atomic:

 $A^* + B_2 \rightarrow AB + B$ 

## Synthesis

Electronic:

 $e + A \rightarrow A^* + e$ 

 $A^{*} + B \rightarrow AB$ 

Atomic:

 $A+B \ \rightarrow \ AB$ 

The generalized equations given above can be exemplified with oxygen and nitrogen as follows,  $O(^{1}D)$ ,  $O(^{3}P)$ ,  $OH^{\bullet}$  are the radicals.

## **Electron impact**

$$e + O_2 \rightarrow O(^{3}P) + O(^{3}P) + e$$

$$e + O_2 \rightarrow O(^{3}P) + O(^{1}D) + e$$

$$e + H_2O \rightarrow OH \cdot + H \cdot + e$$

$$e + N_2 \rightarrow N \cdot + N \cdot + e$$

$$e + N2 \rightarrow N2 \cdot + e$$

$$e + NO \rightarrow N \cdot + O \cdot + e$$

## **Ionization clusters**

$$e + O_2 \rightarrow O_2^+ + e$$
$$O_2^+ \rightarrow O_2^+ (H_2 O)$$
$$O_2^+ (H_2 O) + H_2 O \rightarrow HO_3^+ + O_2 + OH \bullet$$

 $O_2^+(H_2O) + H_2O \rightarrow HO_3^+(OH) + O_2$ 

 $\text{HO}_3^+(\text{OH}) + \text{H}_2\text{O} \rightarrow \text{HO}_3^+ + \text{H}_2\text{O} + \text{OH} \bullet$ 

## Quenching

 $O \bullet + H_2O \rightarrow 2OH \bullet$ 

 $N2 \bullet + O_2 \rightarrow N_2 + O \bullet + O \bullet$ 

## Other

- $H \bullet + O_3 \quad \rightarrow \quad OH \bullet + O_2$
- $\bullet HO_2 + NO \quad \rightarrow \quad OH \bullet + NO_2$
- $H \bullet + O_2 + M \rightarrow \bullet HO_2 + M$

# **APPENDIX B**

Relative Humidity Calculations

Relative Humidity calculations entirely from Huang's work (1996).

## Weight of water collected

Weight of dry  $CaSO_4$  (W<sub>d</sub>) = 156.52 g

Weight of wet  $CaSO_4$  ( $W_w$ ) = 159.00 g

Weight of water collected  $(W_{wf}) = 159 - 156.52$ 

= 2.48 g

#### Weight of Wet air

Air flow rate = 2.5 L/min for 60 min Air flow (volume) = 2.5 x 60 = 150 L Density of air = 1.161 g/L @ 300K, 1bar Weight of wet air ( $W_{af}$ ) = 1.161 \* 150 = 174.15 g

#### **Calculation of relative humidity**

 $RH = [(Wwf/W_{af})/(Saturated content of water in air)] X 100\%$ 

Saturated content of water in air =  $0.02 \text{ g H}_2\text{O/g}$  Air

 $RH = [(2.48/174.15)/0.02] \times 100\%$ 

RH = 71.2%

# **APPENDIX C**

Reynolds Number Calculation

#### Flow rates used

2.5 L/min and 4.5 L/min

## **Dimensions of the reactor**

Height of reactor = 30 cm

Diameter of reactor = 3.1 cm

Area of reactor =  $2\pi r^2$ 

Where,

 $\pi = 3.14$ 

r = radius of the reactor = 1.55 cm

Area of reactor =  $2 \times (3.14) \times (0.6)^2$ 

Area of reactor =  $2.3 \text{ cm}^2$ 

Volume of reactor = 28 mL

At 2.5L/min

According to equation 9

 $N_{Re} = (Dv\rho)/\mu$ 

Flow velocity = 2500/2.3 = 1086.9 cm/min = 18.11 cm/sec

Density of air ( $\rho$ ) = 1.29 Kg/m<sup>3</sup> = 1.29 x 10<sup>-6</sup> Kg/cm<sup>3</sup>

Dynamic viscosity of air ( $\mu$ ) = 1.82 x 10<sup>-5</sup> Kg /m.s

 $N_{Re} = (0.6 \text{ x } 18.11 \text{ x } 1.29 \text{ x } 10^{-6})/(1.82 \text{ x } 10^{-8}) = 770.4$ 

# **APPENDIX D**

Experimental Data

Residence time: 0.4 sec

Power input: 68 watt

Carrier gas: Air

Table D 1. B. subtil	is destrucion data	in air	plasma # B1
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Time	e (min)	Plasma status	Flow path	Bacteri	a survived
Flow	Reactor	(on/off)	(bypass/reactor)	CFU	CFU/L
10	0	off	initial reading	278	31
20	9	on	reactor	0	0
28	16	on	reactor	0	0
36	24	on	reactor	0	0
47	35	off	reactor	194	22
56	43	off	bypass	324	36

CFU/L = CFUs/(flow rate x sampling time)

Residence time: 0.7 sec

Power input: 68 watt

Carrier gas: Air

Table D 2. <i>B</i> .	subtilis	destruction	data in	air	plasma	#B2
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Time	e (min)	Plasma status	Flow path	th Bacteria surviv	
Flow	Reactor	(on/off)	(bypass/reactor)	CFU	CFU/L
10	0	off	initial reading	394	79
20	9	on	reactor	0	0
28	16	on	reactor	0	0
36	23	on	reactor	0	0
47	34	off	reactor	410	82
56	41	off	bypass	456	91

CFU/L = CFUs/(flow rate x sampling time)

Residence time: 0.4 sec

Power input: 38 watt

Carrier gas: Air

Tim	e (min)	(min) Plasma status Flow path		Bacteria survived	
Flow	Reactor	(on/off)	(bypass/reactor)	CFU	CFU/L
10	0	off	initial reading	216	24
20	9	on	reactor	0	0
28	16	on	reactor	0	0
36	23	on	reactor	0	0
47	35	off	reactor	198	22
56	42	off	bypass	283	31

CFU/L = CFUs/(flow rate x sampling time)

Residence time: 0.7 sec

Power input: 38 watt

Carrier gas: Air

Table D 4. B. subtilis	destruction data	a in air plasma #B4
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Time	e (min)	Plasma status	Flow path	Bacteria survived	
Flow	Reactor	(on/off)	(bypass/reactor)	CFU	CFU/L
10	0	off	initial reading	246	49
20	9	on	reactor	0	0
28	16	on	reactor	0	0
36	24	on	reactor	0	0
47	35	off	reactor	224	45
56	42	off	bypass	248	50

CFU/L = CFUs/(flow rate x sampling time)

Residence time: 0.4 sec

Power input: 27 watt

Carrier gas: Air

Time (min)		Plasma status	Flow path	Bacteria survived	
Flow	Reactor	(on/off)	(bypass/reactor)	CFU	CFU/L
10	0	off	initial reading	216	24
20	9	on	reactor	0	0
28	16	on	reactor	0	0
36	24	on	reactor	0	0
46	35	off	reactor	168	19
54	42	off	bypass	191	21

CFU/L = CFUs/(flow rate x sampling time)

Residence time: 0.7 sec

Power input: 27 watt

Carrier gas: Air

Time (min)		Plasma status	Flow path	Bacteria survive	
Flow	Reactor	(on/off)	(bypass/reactor)	CFU	CFU/L
10	0	off	initial reading	254	51
20	10	on	reactor	0	0
28	18	on	reactor	0	0
36	26	on	reactor	0	0
47	37	off	reactor	280	56
56	45	off	bypass	272	54

CFU/L = CFUs/(flow rate x sampling time)

### Residence time: 0.4 sec

Power input: 68 watt

Carrier gas: Air

Time (min)		Plasma status	Flow path	Bacteria survived	
Flow	Reactor	(on/off)	(bypass/reactor)	CFU	CFU/L
10	0	off	initial reading	311	35
19	9	on	reactor	0	0
27	17	on	reactor	0	0
36	25	on	reactor	0	0
47	36	off	reactor	176	20
54	43	off	bypass	TMTC*	TMTC*

Table D 7. E. coli destruction in air plasma #E1

CFU/L = CFUs/(flow rate x sampling time)

\* - TMTC - Too many colony forming units to count

Residence time: 0.7 sec

Power input: 68 watt

Carrier gas: Air

<b>Table D 8.</b> <i>E</i>	. <i>coli</i> destruc	tion data in	air plasma # E2
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Time (min)		Plasma status	Flow path	Bacteria survive	
Flow	Reactor	(on/off)	(bypass/reactor)	CFU	CFU/L
10	0	off	initial reading	176	35
24	12	on	reactor	0	0
32	20	on	reactor	0	0
40	28	on	reactor	0	0
51	39	off	reactor	163	33
61	47	off	bypass	700	140

CFU/L = CFUs/(flow rate x sampling time)

### Residence time: 0.4 sec

Power input: 38 watt

Carrier gas: Air

Time (min)		Plasma status	Flow path	Bacteria survived	
Flow	Reactor	(on/off)	(bypass/reactor)	CFU	CFU/L
10	0	off	initial reading	541	60
19	9	on	reactor	0	0
27	17	on	reactor	0	0
34	24	on	reactor	0	0
45	37	off	reactor	190	21
52	44	off	bypass	TMTC*	TMTC*

CFU/L = CFUs/(flow rate x sampling time)

\* - TMTC – Too many colony forming units to count

### Residence time: 0.7 sec

Power input: 38 watt

Carrier gas: Air

Time (min)		Plasma status	Flow path	Bacteria survived	
Flow	Reactor	(on/off)	(bypass/reactor)	CFU	CFU/L
10	0	off	initial reading	217	43
19	9	on	reactor	0	0
27	17	on	reactor	0	0
36	26	on	reactor	0	0
46	36	off	reactor	157	31
53	43	off	bypass	TMTC*	TMTC*

CFU/L = CFUs/(flow rate x sampling time)

\* - TMTC – Too many colony forming units to count

### Residence time: 0.4 sec

Power input: 27 watt

Carrier gas: Air

Time (min)		Plasma status	Flow path	Bacteria survived	
Flow	Reactor	(on/off)	(bypass/reactor)	CFU	CFU/L
10	0	off	initial reading	217	24
20	9	on	reactor	0	0
27	17	on	reactor	0	0
36	26	on	reactor	0	0
47	36	off	reactor	157	17
55	43	off	bypass	TMTC*	TMTC*

CFU/L = CFUs/(flow rate x sampling time)

\* - TMTC – Too many colony forming units to count

### Residence time: 0.7 sec

Power input: 27 watt

Carrier gas: Air

Time (min)		Plasma status	Flow path	Bacteria	survived
Flow	Reactor	(on/off)	(bypass/reactor)	CFU	CFU/L
10	0	off	initial reading	226	45
20	9	on	reactor	0	0
27	17	on	reactor	0	0
36	24	on	reactor	0	0
47	35	off	reactor	175	35
55	44	off	bypass	TMTC*	TMTC*

CFU/L = CFUs/(flow rate x sampling time)

\* - TMTC – Too many colony forming units to count

Residence time: 0.4 sec

Power input: 68 watt

Carrier gas: Nitrogen

Time (min)		Plasma status	Plasma status Flow path		Bacteria survived	
Flow	Reactor	(on/off)	(bypass/reactor)	CFU	CFU/L	
10	0	off	initial reading	175	19	
20	9	on	reactor	0	0	
27	17	on	reactor	0	0	
36	24	on	reactor	1	0.1	
47	34	off	reactor	83	9	
55	42	off	bypass	TMTC*	TMTC*	

CFU/L = CFUs/(flow rate x sampling time)

\* - TMTC – Too many colony forming units to count

Residence time: 0.7 sec

Power input: 68 watt

Carrier gas: Nitrogen

Time (min)		Plasma status	Flow path	Bacteri	a survived
Flow	Reactor	(on/off)	(bypass/reactor)	CFU	CFU/L
10	0	off	initial reading	291	58
20	9	on	reactor	0	0
28	17	on	reactor	1	0.2
36	25	on	reactor	1	0.2
47	36	off	reactor	199	40
55	44	off	bypass	325	65

Table D 14. B. subtilis destruction data in nitroger	plasma #B8
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CFU/L = CFUs/(flow rate x sampling time)

Residence time: 0.4 sec

Power input: 38 watt

Carrier gas: Nitrogen

Table D 15. B. subtilis	destruction data in nit	trogen plasma #B9
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Tim	e(min)	Plasma status Flow path Bacteria sur		survived	
Flow	Reactor	(on/off)	(bypass/reactor)	CFU	CFU/L
10	0	off	initial reading	180	20
20	9	on	reactor	1	0.1
27	16	on	reactor	1	0.1
36	23	on	reactor	1	0.1
47	33	off	reactor	95	11
55	42	off	bypass	TMTC*	TMTC*

CFU/L = CFUs/(flow rate x sampling time)

\* - TMTC – Too many colony forming units to count

Residence time: 0.7 sec

Power input: 38 watt

Carrier gas: Nitrogen

Time (min)		Plasma status	Flow path Bacte		ia survived	
Flow	Reactor	(on/off)	(bypass/reactor)	CFU	CFU/L	
10	0	off	initial reading	277	31	
20	11	on	reactor	0	0	
27	19	on	reactor	0	0	
36	27	on	reactor	0	0	
47	38	off	reactor	267	30	
55	46	off	bypass	TMTC*	TMTC*	

CFU/L = CFUs/(flow rate x sampling time)

\* - TMTC – Too many colony forming units to count

Residence time: 0.4 sec

Power input: 27 watt

Carrier gas: Nitrogen

Table D 17. <i>B</i> .	. subtilis destruction	data in nitrogen	plasma # B11
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Time (min)		Plasma status	Flow path	Bacteria	survived
Flow	Reactor	(on/off)	(bypass/reactor)	CFU	CFU/L
10	0	off	initial reading	157	17
18	10	on	reactor	0	0
27	18	on	reactor	0	0
36	25	on	reactor	0	0
47	36	off	reactor	162	18
55	45	off	bypass	TMTC*	TMTC*

CFU/L = CFUs/(flow rate x sampling time)

\* - TMTC – Too many colony forming units to count

Residence time: 0.7 sec

Power input: 27 watt

Carrier gas: Nitrogen

Time	(min)	Plasma status	Flow path	Bacteria survived	
Flow	Reactor	(on/off)	(bypass/reactor)	CFU	CFU/L
10	0	off	initial reading	292	58
20	9	on	reactor	1	0.2
27	16	on	reactor	0	0
36	24	on	reactor	0	0
47	35	off	reactor	342	68
55	43	off	bypass	335	67

CFU/L = CFUs/(flow rate x sampling time)

Residence time: 0.7 sec

Power input: 27 watt

Carrier gas: Nitrogen

Table D 19. <i>B</i>	. subtilis destruction	data in nitrogen	plasma #B13
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Time	e (min)	Plasma status	Flow path	Bacteria survived	
Flow	Reactor	(on/off)	(bypass/reactor)	CFU	CFU/L
10	0	off	initial reading	174	35
20	10	on	reactor	0	0
27	18	on	reactor	1	0.2
36	26	on	reactor	1	0.2
47	37	off	reactor	164	33
55	45	off	bypass	315	63

CFU/L = CFUs/(flow rate x sampling time)

Residence time: 0.4 sec

Power input: 68 watt

Carrier gas: Nitrogen

Time (min)		in) Plasma status Flow path		Bacteria survived	
Flow	Reactor	(on/off)	(bypass/reactor)	CFU	CFU/L
10	0	off	initial reading 194		28
20	10	on	reactor	0	0
27	18	on	reactor	0	0
36	26	on	reactor	1	0.1
47	36	off	reactor	113	13
55	44	off	bypass	326	36

Table D 20. B. subtilis destruction data in nitrogen plasma #B14
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CFU/L = CFUs/(flow rate x sampling time)

Residence time: 0.4 sec

Power input: 68 watt

# Carrier gas: Nitrogen

Time	e (min)	Plasma status	Flow path	Bacteria survived	
Flow	Reactor	(on/off)	(bypass/reactor)	CFU	CFU/L
10	0	off	initial reading	228	25
19	11	on	reactor	0	0
26	18	on	reactor	0	0
33	25	on	reactor	0	0
44	36	off	reactor	127	14
52	44	off	bypass	478	53

CFU/L = CFUs/(flow rate x sampling time)

Residence time: 0.7 sec

Power input: 68 watt

Carrier gas: Nitrogen

Time	e (min)	Plasma status	Flow path	Bacteria survived	
Flow	Reactor	(on/off)	(bypass/reactor)	CFU	CFU/L
10	0	off	initial reading	382	76
21	11	on	reactor	0	0
29	19	on	reactor	0	0
35	25	on	reactor	0	0
48	38	off	reactor	86	17
57	47	off	bypass	132	26

CFU/L = CFUs/(flow rate x sampling time)

Residence time: 0.4 sec

Power input: 38 watt

Carrier gas: Nitrogen

Table D 23. E. coli destruction data in nitrogen plasma #E9

Time	e (min)	Plasma status	Flow path	Bacteria survived	
Flow	Reactor	(on/off)	(bypass/reactor)	CFU	CFU/L
10	0	off	initial reading	235	26
20	10	on	reactor	0	0
28	18	on	reactor	0	0
36	27	on	reactor	0	0
48	39	off	reactor	175	19
57	48	off	bypass	646	72

CFU/L = CFUs/(flow rate x sampling time)

Residence time: 0.7 sec

Power input: 38 watt

Carrier gas: Nitrogen

Table D 24.	E.	coli	destruction	data in	nitrogen	plasma #E10
	<b>_</b> .	con	acou action	uata m	mer ogen	

Time	e (min)	Plasma status	Flow path	Bacteria survived	
Flow	Reactor	(on/off)	(bypass/reactor) CI		CFU/L
10	0	off	initial reading	567	113
20	10	on	reactor	0	0
27	17	on	reactor	0	0
36	25	on	reactor	0	0
48	37	off	reactor	313	63
56	45	off	bypass	374	75

CFU/L = CFUs/(flow rate x sampling time)

Residence time: 0.4 sec

Power input: 27 watt

Carrier gas: Nitrogen

Table D 25. <i>E</i> .	<i>coli</i> destruc	tion data in n	nitrogen plasma :	#E11

Time	e (min)	Plasma status	Flow path	Bacte	eria survived
Flow	Reactor	(on/off)	(bypass/reactor)	CFU	CFU/L
10	0	off	initial reading	375	42
21	11	on	reactor	0	0
29	19	on	reactor	0	0
36	26	on	reactor	0	0
48	38	off	reactor	100	11
56	46	off	bypass	199	22

CFU/L = CFUs/(flow rate x sampling time)

Residence time: 0.7 sec

Power input: 27 watt

Carrier gas: Nitrogen

Time	e (min)	Plasma status	Flow path	Bacte	eria survived
Flow	Reactor	(on/off)	(bypass/reactor)	CFU	CFU/L
10	0	off	initial reading	478	96
21	11	on	reactor	0	0
28	19	on	reactor	0	0
36	27	on	reactor	0	0
48	39	off	reactor	305	61
56	47	off	bypass	374	75

CFU/L = CFUs/(flow rate x sampling time)

Residence time: 0.4 sec

# Power input: 68 watt

### Carrier gas: air with Carbon particulates

Tim	e (min)	Plasma status	Flow path	Particulate Conc.	Bacteri	a survived
Flow	Reactor	(on/off)	(bypass/ reactor)	$\mu g/m^3$	CFU	CFU/L
10	0	off	initial reading		194	24
18	8	off	reactor	48000		
30	20	on	reactor		0	0
39	29	on	reactor		0	0
47	37	on	reactor		0	0
55	45	off	reactor		197	22
67	57	off	reactor	64000		
81	71	off	bypass		254	28

Table D 27. B. subtilis destruction data with particulate	s #B15
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CFU/L = CFUs/(flow rate x sampling time)

Residence time: 0.7 sec

Power input: 68 watt

# Carrier gas: air with carbon particulates

Tim	e (min)	Plasma status	Flow path	Particulate Conc.	Bacteri	a survived
Flow	Reactor	(on/off)	(bypass /reactor)	$\mu g/m^3$	CFU	CFU/L
10	0	off	initial reading		189	38
18	8	off	reactor	56000		
31	21	on	reactor		0	0
39	29	on	reactor		0	0
47	37	on	reactor		0	0
55	45	off	reactor		114	23
67	59	off	reactor	64000		
79	72	off	bypass		237	47

Table D 28. B. subtilis destruction with particular	rticulates #B16
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CFU/L = CFUs/(flow rate x sampling time)

#### Residence time: 0.4 sec

# Power input: 68 watt

# Carrier gas: air with carbon particulates

Tim	e (min)	Plasma status	Flow path	Particulate Conc.	Bacteri	a survived
Flow	Reactor	(on/off)	(bypass/ reactor)	$\mu g/m^3$	CFU	CFU/L
10	0	off	initial reading		232	26
17	7	off	reactor	56000		
31	21	on	reactor		0	0
39	29	on	reactor		0	0
47	37	on	reactor		0	0
55	45	off	reactor		317	35
68	58	off	reactor	72000		
80	70	off	bypass		352	39

Table D 29. B. subtilis destruction data with particulates #B17
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CFU/L = CFUs/(flow rate x sampling time)

Residence time: 0.7 sec

# Power input: 38 watt

# Carrier gas: air with Carbon particulates

Tim	e (min)	Plasma status	Flow path	Particulate Conc.	Bacteri	a survived
Flow	Reactor	(on/off)	(bypass/ reactor)	$\mu g/m^3$	CFU	CFU/L
10	0	off	initial reading		187	37
18	8	off	reactor	72000		
33	23	on	reactor		0	0
41	31	on	reactor		0	0
48	38	on	reactor		0	0
56	46	off	reactor		137	27
68	58	off	reactor	72000		
81	71	off	bypass		224	45

Table D 30. B. subtilis destruction	data wi	th particulates #B18
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CFU/L = CFUs/(flow rate x sampling time)

Residence time: 0.4 sec

Power input: 27 watt

# Carrier gas: air with Carbon particulates

Time (min)		Plasma status	Flow path	Particulate Conc.	Bacteria survived	
Flow	Reactor	(on/off)	(bypass/ reactor)	$\mu g/m^3$	CFU	CFU/L
10	0	off	initial reading		267	26
17	7	off	reactor	35,556		
30	20	on	reactor		0	0
40	30	on	reactor		0	0
48	38	on	reactor		0	0
57	47	off	reactor		224	25
66	59	off	reactor	35,556		
79	72	off	bypass		383	43

Table D 31. B. subtilis destruction data with particulates #B19
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CFU/L = CFUs/(flow rate x sampling time)

Residence time: 0.7 sec

Power input: 27 watt

# Carrier gas: air with Carbon particulates

Time (min)		Plasma status	Flow path	Particulate Conc.	Bacteria survive	
Flow	Reactor	(on/off)	(bypass/ reactor)	$\mu g/m^3$	CFU	CFU/L
10	0	off	initial reading		203	41
18	8	off	reactor	48000		
30	20	on	reactor		0	0
38	28	on	reactor		0	0
45	35	on	reactor		0	0
53	43	off	reactor		158	32
64	54	off	reactor	64000		
78	68	off	bypass		319	64

Table D 32. B. subtilis destruction with particul	ates #B20
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CFU/L = CFUs/(flow rate x sampling time)

#### Residence time: 0.4 sec

### Power input: 68 watt

### Carrier gas: air with Carbon particulates

Time (min)		Plasma status	Flow path	Particulate Conc.	Bacteria survive	
Flow	Reactor	(on/off)	(bypass/ reactor)	$\mu g/m^3$	CFU	CFU/L
10	0	off	initial reading		502	56
18	8	off	reactor	64000		
30	20	on	reactor		0	0
38	28	on	reactor		0	0
46	36	on	reactor		0	0
57	47	off	reactor		261	29
69	59	off	reactor	80000		
81	71	off	bypass		TMTC*	TMTC*

CFU/L = CFUs/(flow rate x sampling time)

\* - TMTC – Too many colony forming units to count

### Residence time: 0.7 sec

# Power input: 68 watt

### Carrier gas: air with Carbon particulates

Table D	Table D 54. L. Cou destruction data with particulates #E14								
Time (min)		Plasma status	Flow path	Particulate Conc.	Bacteria survived				
Flow	Reactor	(on/off)	(bypass/ reactor)	µg/m <sup>3</sup>	CFU	CFU/L			
10	0	off	initial reading		298	60			
18	8	off	reactor	64,000					
30	20	on	reactor		0	0			
38	28	on	reactor		0	0			
46	36	on	reactor		0	0			
54	44	off	reactor		139	28			
67	57	off	reactor	56,000					
79	69	off	bypass		586	117			

CFU/L = CFUs/(flow rate x sampling time)

#### Residence time: 0.4 sec

### Power input: 38 watt

### Carrier gas: air with Carbon particulates

Time (min)		Plasma status	Flow path	Particulate Conc.		teria ived
Flow	Reactor	(on/off)	(bypass/ reactor)	$\mu g/m^3$	CFU	CFU/L
10	0	off	initial reading		353	39
18	8	off	reactor	72000		
32	22	on	reactor		0	0
40	30	on	reactor		0	0
48	38	on	reactor		0	0
54	45	off	reactor		202	22
67	57	off	reactor	64000		
79	69	off	bypass		TMTC*	TMTC*

Table D 35. <i>E</i> .	coli destruction	data with	particulates #E15
			1

CFU/L = CFUs/(flow rate x sampling time)

\* - TMTC – Too many colony forming units to count

#### Residence time: 0.7 sec

### Power input: 38 watt

### Carrier gas: air with Carbon particulates

Time (min)		Plasma status	Flow path	Particulate Conc.	Bacteria survive	
Flow	Reactor	(on/off)	(bypass/ reactor)	$\mu g/m^3$	CFU	CFU/L
10	0	off	initial reading		387	77
18	8	off	reactor	64000		
30	20	on	reactor		0	0
38	28	on	reactor		0	0
45	35	on	reactor		0	0
54	43	off	reactor		151	30
67	55	off	reactor	80000		
79	68	off	bypass		TMTC*	TMTC*

CFU/L = CFUs/(flow rate x sampling time)

\* - TMTC – Too many colony forming units to count

### Residence time: 0.4 sec

# Power input: 27 watt

# Carrier gas: air with Carbon particulates

Time (min)		Plasma status	Flow path	Particulate Conc.	Bacteria survive	
Flow	Reactor	(on/off)	(bypass/ reactor)	µg/m <sup>3</sup>	CFU	CFU/L
10	0	off	initial reading		567	63
18	8	off	reactor	26,667		
30	20	on	reactor		0	0
38	28	on	reactor		0	0
46	36	on	reactor		0	0
54	44	off	reactor		378	42
66	56	off	reactor	35,556		
78	68	off	bypass		700	78

CFU/L = CFUs/(flow rate x sampling time)

Residence time: 0.7 sec

Power input: 27 watt

# Carrier gas: air with Carbon particulates

<b>i</b>							
Time (min)		Reactor Status	Flow path	Particulate Conc.	Bacteria survived		
Flow	Reactor	(on/off)	(bypass/ reactor) µg/m <sup>3</sup>		CFU	CFU/L	
10	0	off	initial reading		87	17	
18	8	off	reactor	56000			
30	20	on	reactor		0	0	
38	28	on	reactor		0	0	
46	36	on	reactor		0	0	
54	44	off	reactor		67	13	
66	56	off	reactor	64000			
78	68	off	bypass		139	28	

Table D 38. E. coli destruction	ı data with particulates #E18
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CFU/L = CFUs/(flow rate x sampling time)

#### Residence time: 0.7 sec

Power input: 60 watt

### Carrier gas: air

Time (min)		Plasma status	Flow path Bacteria s		survived
Flow	Reactor	(on/off)	(bypass/reactor)	CFU	CFU/L
10	0	off	1 min plasma equilibrium time Bypass	TMTC*	TMTC*
19	9	on	2 min plasma equilibrium time through reactor	1	0.08
25	15	on	reactor	0	0
31	21	on	reactor	3	0.2
38	28	off	Plasma off for 0.5 min - reactor	TMTC*	TMTC*
46	36	off	bypass	TMTC*	TMTC*

CFU/L = CFUs/(flow rate x sampling time)

\* - TMTC – Too many colony forming units to count

Data obtained from Schroeder's (1996) work

#### Residence time: 0.4 sec

Power input: 60 watt

### Carrier gas: air

Table D 40. <i>B</i>	. subtilis	destruction #SB2

Time (min)		Plasma status	Flow path Bacteria sur		survived
Flow	Reactor	(on/off)	(bypass/reactor)	CFU	CFU/L
10	0	off	0.5 min plasma equilibrium time Bypass	TMTC*	TMTC*
18	8	on	1 min plasma equilibrium time through reactor	9	0.4
24	14	on	reactor	1	0.04
32	22	on	reactor	1	0.04
39	29	off	Plasma off for 1 min - reactor	TMTC*	TMTC*
46	36	off	bypass	TMTC*	TMTC*

CFU/L = CFUs/(flow rate x sampling time)

\* - TMTC – Too many colony forming units to count

Data obtained from Schroeder's (1996) work

#### Residence time: 0.4 sec

Power input: 34.8 watt

### Carrier gas: air

Table D 41. <i>B. subtilis</i> destruction #SE
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Time (min)		Plasma status	Flow path Ba		Bacteria survived	
Flow	Reactor	(on/off)	(bypass/reactor)	CFU	CFU/L	
10	0	off	1 min plasma equilibrium time Bypass	TMTC*	TMTC*	
19	9	on	1 min plasma equilibrium time through reactor	3	0.1	
25	15	on	reactor	3	0.1	
31	21	on	reactor	4	0.2	
38	28	off	Plasma off for 0.5 min - reactor	TMTC*	TMTC*	
46	36	off	bypass	TMTC*	TMTC*	

CFU/L = CFUs/(flow rate x sampling time)

\* - TMTC – Too many colony forming units to count

Data obtained from Schroeder's (1996) work

## Residence time: 0.7 sec

Power input: 34.8 watt

## Carrier gas: air

Table D 42.	<b>B</b> .	subtilis	destruction #SB4	
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Time	(min)	Plasma	Flow noth		Bacteria s	survive	ł
1 11110 (	(11111)	status	Flow path	24 h	ours	4	8 hours
Flow	Reactor	(on/off)	(bypass/reactor)	CFU	CFU/L	CFU	CFU/L
10	0	off	1 min plasma equilibrium time Bypass	316	63	328	66
18	8	on	1 min plasma equilibrium time through reactor	0	0	0	0
24	14	on	reactor	0	0	0	0
32	22	on	reactor	0	0	0	0
39	29	off	Plasma off for 1 min - reactor	201	40	204	41
46	36	off	bypass	212	42	233	47

CFU/L = CFUs/(flow rate x sampling time)

#### Residence time: 0.4 sec

Power input: 34.8 watt

Carrier gas: air

#### Table D 43. B. subtilis destruction #SB5

Tim	e (min)	Plasma	Flow noth		Bacteria s	survived	
1 11 11		status	Flow path	24 ]	hours	48	hours
Flow	Reactor	(on/off)	(bypass/reactor)	CFU	CFU/L	CFU	CFU/L
10	0	off	1 min plasma equilibrium time Bypass	148	16	144	16
19	9	on	1 min plasma equilibrium time through reactor	0	0	0	0
26	16	on	reactor	1	0.1	1	0.1
32	22	on	reactor	1	0.1	1	0.1
40	30	off	Plasma off for 1 min - reactor	58	6	60	7
49	39	off	bypass	TMTC	TMTC	TMTC	TMTC

CFU/L = CFUs/(flow rate x sampling time)

TMTC – Too many colony forming units to count

#### Residence time: 0.4 sec

Power input: 60 watt

#### Carrier gas: air

Tim	a (min)	Plasma	Elow esth		Bacteria s	survived	
1 Im	e (min)	status	Flow path	24 ]	hours	48	hours
Flow	Reactor	(on/off)	(bypass/reactor)	CFU	CFU/L	CFU	CFU/L
10	0	off	1 min plasma equilibrium time Bypass	TMTC	TMTC	TMTC	TMTC
19	9	on	1 min plasma equilibrium time through reactor	0	0	0	0
26	16	on	reactor	0	0	0	0
34	24	on	reactor	0	0	0	0
41	31	off	Plasma off for 1 min - reactor	217	24	210	23
50	40	off	bypass	TMTC	TMTC	TMTC	TMTC

#### Table D 44. B. subtilis destruction #SB6

CFU/L = CFUs/(flow rate x sampling time)

TMTC - Too many colony forming units to count

#### Residence time: 0.4 sec

Power input: 60 watt

## Carrier gas: air

Tim	a (min)	Plasma	Elever moth		Bacteria s	survived	
1 III	e (min)	status	Flow path	24 ]	hours	48	hours
Flow	Reactor	(on/off)	(bypass/reactor)	CFU	CFU/L	CFU	CFU/L
10	0	off	1 min plasma equilibrium time Bypass	138	15	129	14
18	8	on	1 min plasma equilibrium time through reactor	0	0	0	0
26	16	on	reactor	0	0	0	0
34	24	on	reactor	0	0	0	0
41	31	off	Plasma off for 1 min - reactor	68	8	74	8
51	41	off	bypass	376	42	376	42

#### Table D 45. *B. subtilis* destruction #SB7

CFU/L = CFUs/(flow rate x sampling time)

#### Residence time: 0.4 sec

Power input: 34.8 watt

#### Carrier gas: air

Tim	a (min)	Plasma	Elever a eth		Bacteria s	survived	
1 Im	e (min)	status	Flow path	24 ]	hours	48	hours
Flow	Reactor	(on/off)	(bypass/reactor)	CFU	CFU/L	CFU	CFU/L
10	0	off	1 min plasma equilibrium time Bypass	146	16	152	17
18	8	on	1 min plasma equilibrium time through reactor	0	0	0	0
25	15	on	reactor	0	0	0	0
32	22	on	reactor	0	0	0	0
39	29	off	Plasma off for 1 min - reactor	89	10	87	10
48	38	off	bypass	TMTC	TMTC	TMTC	TMTC

Table D 46. B. subtilis destruction #SB8

CFU/L = CFUs/(flow rate x sampling time)

TMTC - Too many colony forming units to count

## Residence time: 0.7 sec

Power input: 34.8 watt

## Carrier gas: air

Tim	a (min)	Plasma	Elever moth		Bacteria s	survived	
1 Im	e (min)	status	Flow path	24 ]	hours	48	hours
Flow	Reactor	(on/off)	(bypass/reactor)	CFU	CFU/L	CFU	CFU/L
10	0	off	1 min plasma equilibrium time Bypass	202	40	217	43
19	9	on	1 min plasma equilibrium time through reactor	0	0	0	0
27	17	on	reactor	0	0	0	0
35	25	on	reactor	0	0	0	0
42	32	off	Plasma off for 1 min - reactor	219	44	214	43
49	39	off	bypass	207	41	214	43

#### Table D 47. B. subtilis destruction #SB9

CFU/L = CFUs/(flow rate x sampling time)

## Residence time: 0.7 sec

Power input: 30 watt

## Carrier gas: air

Table D 48. B	. subtilis	destruction #S	B10
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Tim	e (min)	Plasma	Flow path		Bacteria s	urvived	
1 1111		status	riow path	24 ]	hours	48	hours
Flow	Reactor	(on/off)	(bypass/reactor)	CFU	CFU/L	CFU	CFU/L
10	0	off	1 min plasma equilibrium time Bypass	238	48	231	46
18	8	on	1 min plasma equilibrium time through reactor	0	0	0	0
26	16	on	reactor	1	0.2	1	0.2
33	23	on	reactor	0	0	0	0
39	29	off	Plasma off for 1 min - reactor	215	43	212	42
48	38	off	bypass	249	50	239	48

CFU/L = CFUs/(flow rate x sampling time)

Residence time: 0.7 sec

Power input: 49.2 watt

## Carrier gas: air

Time (min)		Plasma status	Flow path	Bacteria survived CFU/L		
Flow	Reactor	(on/off)	(bypass/reactor)	CFU	CFU/L	
10	0	off	bypass	2920	234	
18	8	on	reactor	0	0	
24	14	on	reactor	0	0	
32	22	on	Plasma off for 1 min - reactor	1420	114	
41	31	off	bypass	4911	393	

CFU/L = CFUs/(flow rate x sampling time)

#### Residence time: 0.4 sec

Power input: 49.2 watt

## Carrier gas: air

Table D 50. E. coli destruction #S
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Time (min)		Plasma status	Flow path	Bacteria survived	
Flow	Reactor	(on/off)	(bypass/reactor)	CFU	CFU/L
10	0	off	1 min plasma equilibrium time bypass	30	1.3
18	8	on	2 min plasma equilibrium time through reactor	0	0
25	15	on	reactor	0	0
32	22	on	reactor	0	0
38	28	off	Plasma off for 0.5 min - reactor	70	3.1
47	37	off	bypass	480	21.3

CFU/L = CFUs/(flow rate x sampling time)

#### Residence time: 0.4 sec

Power input: 49.2 watt

## Carrier gas: air

Table D 51. E. coli destruction #SE
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Time (	(min)	Plasma status Flow path		Bacteria survived	
Flow	Reactor	(on/off)	(bypass/reactor)	CFU	CFU/L
10	0	off	1 min plasma equilibrium time bypass	TMTC*	TMTC*
19	9	on	2 min plasma equilibrium time through reactor	0	0
25	15	on	reactor	0	0
32	22	on	reactor	7	0.3
41	31	off	bypass	TMTC*	TMTC*

CFU/L = CFUs/(flow rate x sampling time)

\* - TMTC – Too many colony forming units to count

# APPENDIX E

Sample Calculation for Secondary Current, Secondary Power and Energy Density

#### **Secondary Current Calculation**

Volume of reactor = 0.028 L

Primary Voltage = 110 v

Variac setting = 99 v

Secondary Voltage = 12,500 v

Transformation ratio,  $K_N$  = Secondary Voltage/Variac setting = 12,500/99

 $K_{\rm N} = 127$ 

Primary Current,  $I_P = 0.5 A$ 

Assuming current error % at 25 %

Current error (%) = 100 (K<sub>N</sub> I<sub>S</sub> - I<sub>P</sub>) / I<sub>P</sub>

 $25 = 100 (127 \text{ x I}_{\text{S}} - 0.5)/0.5$ 

 $I_S = 0.0049 A$ 

 $I_S = 4.9 \text{ mA}$ 

#### **Secondary Power Calculation**

Secondary Power,  $P_S$  = Secondary Current x Secondary Voltage

 $P_{\rm S} = 0.0049 \text{ x } 12,500$ 

 $P_{\rm S} = 61.8$  watt

#### **Energy Density Calculation**

Residence Time = 0.4 sec

Energy Density, E<sub>D</sub>= (Secondary Power x Residence Time)/ Volume of reactor

 $E_D = (61.8 \text{ x } 0.4)/0.028 = 882.1 \text{ J/L}$ 

# **APPENDIX F**

Error Percentages for Huang's (1996) and Schroeder's (1996) data

s.no	Initial reading (Ir)	Reactor-off (Ro)	Bypass (Bp)	%error reactor- off	% error bypass
no units	CFU/L	CFU/L	CFU/L	(Ir – Ro)x100/Ir	(Ir – Bp)x100/Ir
B1	31	22	36	29.0	-16.1
B2	79	82	91	-3.8	-15.2
В3	24	22	31	8.3	-29.2
B4	49	45	50	8.2	-2.0
В5	24	19	21	20.8	12.5
B6	51	56	54	-9.8	-5.9
			Average Error %	8.8	-9.3

Table F 1. Error % for Huang's (1996) air plasma runs with B. subtilis

s.no	Initial reading (Ir)	Reactor-off (Ro)	Bypass (Bp)	% reactor-off	% bypass
no units	CFU/L	CFU/L	CFU/L	(Ir – Ro)x100/Ir	(Ir – Bp)x100/Ir
E1	35	20	TMTC	42.9	
E2	35	33	140	5.7	-300.0
E3	60	21	TMTC	65.0	
E4	43	31	TMTC	27.9	
E5	24	17	TMTC	29.2	
E6	45	35	TMTC	22.2	
	·		Average %	32.1	-300.0

Table F 2. Error % for Huang's (1996) air plasma runs with *E. coli* 

s.no	Initial reading (Ir)	Reactor-off (Ro)	Bypass (Bp)	% reactor-off	% bypass
no units	CFU/L	CFU/L	CFU/L	(Ir – Ro)x100/Ir	(Ir-Bp)x100/Ir
B7	19	9	TMTC	52.6	
B8	58	40	65	31.0	-12.1
B9	20	11	TMTC	45.0	
B10	31	30	TMTC	3.2	
B11	17	18	TMTC	-5.9	
B12	58	68	67	-17.2	-15.5
			Average %	18.1	-13.8

Table F 3. Error % for Huang's (1996) nitrogen plasma runs with B. subtilis

s.no	Initial reading (Ir)	Reactor-off (Ro)	Bypass (Bp)	% reactor-off	% bypass
no units	CFU/L	CFU/L	CFU/L	(Ir – Ro)x100/Ir	(Ir – Bp)x100/Ir
E7	25	14	53	44.0	-112.0
E8	76	17	26	77.6	65.8
E9	26	19	72	26.9	-176.9
E10	113	63	75	44.2	33.6
E11	42	11	22	73.8	47.6
E12	96	61	75	36.5	21.9
			Average %	50.5	-20.0

Table F 4. Error % for Huang's (1996) nitrogen plasma runs with E. coli

s.no	Initial reading (Ir)	Reactor-off (Ro)	Bypass (Bp)	% reactor-off	% bypass
no units	CFU/L	CFU/L	CFU/L	(Ir – Ro)x100/Ir	(Ir-Bp)x100/Ir
B15	24	22	28	8.3	-16.7
B16	38	23	47	39.5	-23.7
B17	26	35	39	-34.6	-50.0
B18	37	27	45	27.0	-21.6
B19	26	25	43	3.8	-65.4
B20	41	32	64	22.0	-56.1
			Average %	11.0	-38.9

Table F 5. Error % for Huang's (1996) air-particulates plasma runs with B. subtilis

s.no	Initial reading (Ir)	Reactor-off (Ro)	Bypass (Bp)	% reactor-off	% bypass
no units	CFU/L	CFU/L	CFU/L	(Ir – Ro)x100/Ir	(Ir – Bp)x100/Ir
E13	56	29	TMTC	48.2	
E14	60	28	117	53.3	-95.0
E15	39	22	TMTC	43.6	
E16	77	30	TMTC	61.0	
E17	63	42	78	33.3	-23.8
E18	17	13	28	23.5	-64.7
			Average %	43.8	-61.2

Table F 6. Error % for Huang's (1996) air-particulates plasma runs with E. coli

s.no	Initial reading (Ir)	Reactor-off (Ro)	Bypass (Bp)	% reactor-off	% bypass
no units	CFU/L	CFU/L	CFU/L	(Ir – Ro)x100/Ir	(Ir – Bp)x100/Ir
SB4	63	40	42	36.5	33.3
SB5	16	6	TMTC	62.5	
SB6	TMTC	24	TMTC		
SB7	15	8	42	46.7	-180.0
SB8	16	10	TMTC	37.5	
SB9	40	44	41	-10.0	-2.5
SB10	48	43	50	10.4	-4.2
			Average %	30.6	-38.3

Table F 7 Error % corresponding to Schroeder's (1996) data *B. subtilis* - after 24 hrs

s.no	Initial reading (Ir)	Reactor-off (Ro)	Bypass (Bp)	% reactor-off	% bypass
no units	CFU/L	CFU/L	CFU/L	(Ir – Ro)x100/Ir	(Ir – Bp)x100/Ir
SB4	66	41	47	37.88	28.8
SB5	16	7	TMTC	56.25	
SB6	TMTC	23	TMTC		
SB7	14	8	42	42.86	-200.0
SB8	17	10	TMTC	41.18	
SB9	43	43	43	0.00	0.0
SB10	46	42	48	8.70	-4.3
			Average %	31.14	-43.9

Table F 8 Error % corresponding to Schroeder's (1996) data B. subtilis - after 48 hrs

s.no	Initial reading (Ir)	Reactor-off (Ro)	Bypass (Bp)	% reactor-off	% bypass
no units	CFU/L	CFU/L	CFU/L	(Ir – Ro)x100/Ir	(Ir – Bp)x100/Ir
SE1	234	114	393	51.3	-67.9
SE2	1.3	3.1	21.3	-138.5	-1538.5
SE3	TMTC	0.3	TMTC		
	<u>.</u>		Average %	-43.6	-803.2

Table F 9 Error % corresponding to Schroeder's (1996) data for *E. coli* 

s no	Initial reading (Ir)	Reactor-off (Ro)	Bypass (Bp)	% reactor-off	% bypass
no units	CFU/L	CFU/L	CFU/L	(Ir – Ro)x100/Ir	(Ir-Bp)x100/Ir
C1	148	58	TMTC 60.8		
C2	288	156	184	45.8	36.1
C3	316	201	212	36.4	32.9
C5	TMTC	217	TMTC		
C6	197	173	176	12.2	10.7
C7	138	68	376	50.7	-172.5
C8	146	89	TMTC	39.0	
C9	202	219	207	-8.4	-2.5
C10	39				
C11	238	215	249	9.7	-4.6
			Average %	30.8	-16.6

 Table F 10 Error % corresponding to Huang's (1996) verification data

# APPENDIX G

Huang's Verification Data

Solution Volume: 400 mL

Secondary Voltage: 9,500 volt

Flow rate: 4.5 L/min

Variac: 72.5 volt

Date: 6/15/1996

Time: 3:30 pm

Sample No.	Exposure Time	Plasma status	Clock time	Flow path	Bacteria s CFU [CFUs/(flo sampling	J/L ow rate x
	min	(on/off)		(bypass/reactor)	24 hours	48 hours
C1-1	2	off	3:40	initial reading	16	16
C1-2	2	on	3:49	Reactor	0	0
C1-3	2	on	3:55	Reactor	0.1	0.1
C1-4	2	on	4:01	Reactor	0.1	0.1
C1-5	2	off	4:07	Reactor	6	7
C1-6	2	off	4:15	bypass	TMTC	TMTC

Solution Volume: 400 mL

Secondary Voltage: 12,500 volt

Flow rate: 2.5 L/min

Variac: 98 volt

Date: 6/16/1996

Time: 4:20 pm

Sample No.	Exposure Time	Plasma status	Clock time	Flow path	Bacteria survived CFU/L [CFUs/(flow rate x sampling time)]	
	min	(on/off)		(bypass/reactor)	24 hours	48 hours
C2-1	2	off	4:51	initial reading	58	57
C2-2	2	on	5:00	reactor	0	0.2
C2-3	2	on	5:05	reactor	0	0
C2-4	2	on	5:10	reactor	0	0
C2-5	2	off	5:17	reactor	31	34
C2-6	2	off	5:22	bypass	37	49

Solution Volume: 400 mL

Secondary Voltage: 9,500 volt

Flow rate: 2.5 L/min

Variac: 74 volt

Date: 6/17/1996

Time: 5:20 pm

#### Table G 3. Preliminary destruction data in air plasma #C3

Sample No.	Exposure Time	Plasma status	Clock time	Flow path	Bacteria survived CFU/L [CFUs/(flow rate x sampling time)]	
	min	(on/off)		(bypass/reactor)	24 hours	48 hours
C3-1	2	off	5:55	Initial reading	63	66
C3-2	2	on	6:03	reactor	0	0
C3-3	2	on	6:09	reactor	0	0
C3-4	2	on	6:15	reactor	0	0
C3-5	2	off	6:25	reactor	40	41
C3-6	2	off	6:31	bypass	42	47

Solution Volume: 400 mL

Secondary Voltage: 12,500 volt

Flow rate: 4.5 L/min

Variac: 74 volt

Date: 6/191996

Time: 8:00pm

#### Table G 4. Preliminary destruction data in air plasma #C5

Sample No.	Exposure Time	Plasma status	Clock time	Flow path	Bacteria survived CFU/L [CFUs/(flow rate x sampling time)]	
	min	(on/off)		(bypass/reactor)	24 hours	48 hours
C5-1	2	off	8:35	initial reading	TMTC	TMTC
C5-2	2	on	8:55	reactor	0	0
C5-3	2	on	9:04	reactor	0	0
C5-4	2	on	9:09	reactor	0	0
C5-5	2	off	9:17	reactor	24	24
C5-6	2	off	9:23	bypass	TMTC	TMTC

Solution Volume: 400 mL

Secondary Voltage: 12,500 volt

Flow rate: 2.5 L/min

Variac: 99 volt

Date: 6/21/1996

Time: 5:20 pm

#### Table G 5. Preliminary destruction data in air plasma #C6

Sample No.	Exposure Time	Plasma status	Clock time	Flow path	Bacteria s CFU [CFUs/(flo sampling	J/L ow rate x
	min	(on/off)		(bypass/reactor)	24 hours	48 hours
C6-1	2	off	5:53	initial reading	39	39
C6-2	2	on	6:04	reactor	0	0
C6-3	2	on	6:10	reactor	0	0
C6-4	2	on	6:15	reactor	0.2	0.2
C6-5	2	off	6:20	reactor	35	33
C6-6	2	off	6:26	bypass	35	34

Solution Volume: 450 mL

Secondary Voltage: 12,500 volt

Flow rate: 4.5 L/min

Variac: 99 volt

Date: 6/22/1996

Time: 5:10 pm

Sample No.	Exposure Time	Plasma status	Clock time	Flow path	Bacteria s CFU [CFUs/(flo sampling	J/L ow rate x
	min	(on/off)		(bypass/reactor)	24 hours	48 hours
C7-1	2	off	5:56	initial reading	15	14
C7-2	2	on	6:05	reactor	0	0
C7-3	2	on	6:09	reactor	0	0
C7-4	2	on	6:15	reactor	0	0
C7-5	2	off	6:23	reactor	8	8
C7-6	2	off	6:31	bypass	412	42

Solution Volume: 450 mL

Secondary Voltage: 9,500 volt

Flow rate: 4.5 L/min

Variac: 75 volt

Date: 6/23/1996

Time: 5:30 pm

#### Table G 7. Preliminary destruction data in air plasma #C8

Sample No.	Exposure Time	Plasma status	Clock time	Flow path	Bacteria survived CFU/L [CFUs/(flow rate x sampling time)]	
	min	(on/off)		(bypass/reactor)	24 hours	48 hours
C8-1	2	off	6:19	Initial reading	16	17
C8-2	2	on	6:28	reactor	0	0.2
C8-3	2	on	6:36	reactor	0	0
C8-4	2	on	6:42	reactor	0	0
C8-5	2	off	6:50	reactor	10	10
C8-6	2	off	6:56	bypass	TMTC	TMTC

Secondary Voltage: 12,500 volt

Flow rate: 2.5 L/min

Variac: 98 volt

Date: 6/26/1996

Time: 8:00 pm

## Table G 8. Preliminary destruction data in air plasma #C9

Sample No.	Exposure Time	Plasma status	Clock time	Flow path	Bacteria survived CFU/L [CFUs/(flow rate x sampling time)]	
	min	(on/off)		(bypass/reactor)	24 hours	48 hours
C9-1	2	off	8:35	initial reading	40	43
C9-2	2	on	8:44	reactor	0	0
С9-3	2	on	8:50	reactor	0	0
C9-4	2	on	8:56	reactor	0	0
C9-5	2	off	9:06	reactor	44	43
С9-6	2	off	9:12	bypass	41	43

Solution Volume: 450 mL

Secondary Voltage: 12,500 volt

Flow rate: 2.5 L/min

Variac: 60 volt

Date: 7/1/1996

Time: 7:45 pm

#### Table G 9. Preliminary destruction data in air plasma #C11

Sample No.	Exposure Time	Plasma status	Clock time	Flow path	Bacteria survived CFU/L [CFUs/(flow rate x sampling time)]	
	min	(on/off)		(bypass/reactor)	24 hours	48 hours
C11-1	2	Off	8:23	initial reading	48	46
C11-2	2	On	8:32	reactor	0	0
C11-3	2	On	8:38	reactor	0.2	0.2
C11-4	2	On	8:44	reactor	0	0
C11-5	2	Off	8:50	reactor	43	42
C11-6	2	Off	8:58	bypass	50	48

Solution Volume: 450 mL

Secondary Voltage: 9,500 volt

Flow rate: 4.5 L/min

Date: 6/12/1996

Time: 7:30 pm

Sample No.	Exposure Time	Plasma status	Flow path	Bacteria survived CFU/L [CFUs/(flow rate x sampling time)]	
	min	(on/off)	(bypass/reactor)	24 hours	48 hours
B1	0.5	Off	Bypass	19	18
B2	1	Off	Bypass	42	38
B3	2	Off	Bypass	24	24
B4	3	Off	Bypass	20	ND*

\*ND – Not Determined

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