Chapter 3

Methodology & Experimental Setup

A parallel plate dielectric barrier discharge (DBD) system was constructed for the purpose of bacterial inactivation application. The system consisted of a high voltage power supply, a pair of electrodes, and dielectric sheet to act as the barrier. A high voltage probe, current probe, spectrometer and an oscilloscope were employed as the monitoring/diagnostic tools for the DBD system.

3.1 Dielectric Barrier Discharge System Setup

3.1.1 Electrodes

A set of plane-parallel electrodes was designed and used in this DBD system. The electrodes were two flat round copper plates which measure 3.8cm in diameter and 1cm thick (Figure 3.1). The edge of the discs is rounded to avoid high electric field at the sharp bend. Each copper plate is supported by a copper rod, measuring 1cm in diameter and 8cm long, which is screwed into the centre at one side of the plate.

The electrodes are placed in a Teflon insulator holder. One of the electrodes was fastened (immovable) onto the base of a travelling microscope, while the other was mounted onto a slider with Vernier scale. This enables the gap between the electrodes to be adjusted in steps of 0.01mm. One of the electrodes was powered by high voltage power supply and the other electrode was connected to ground (movable one).

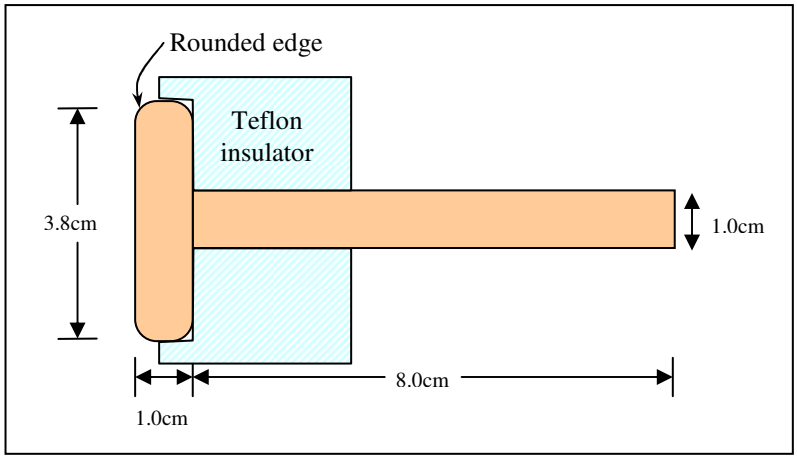

Figure 3.1: Dimensions of the electrode.

3.1.2 The dielectric barrier

The type of dielectric material is one of the key parameters to the behaviour of the barrier discharge. Two types of dielectric materials were used in this project. The first one was a glass sheet of thickness 2 mm, and the second was an alumina (aluminum oxide) sheet with thickness of 1mm. The dielectric constant of the glass sheet is 7.5 (Kaye and Laby, 1995); and the dielectric constant for alumina sheet is 9.0 (ε_r = 9.0) (*Alumina Material Information provided by the manufacturer*). The dielectric sheet was arranged to cover one of the electrodes only; and this electrode was the powered high voltage electrode in most of the cases.

3.1.3 Air gap

The air gap is the region where the barrier discharge is established. This air gap is defined by the space between the dielectric sheet and the grounded electrode; the dielectric sheet being in contact with the powered electrode. This configuration is similar to that in Figure 2.1(a).

The capacitance of the parallel-plate electrodes DBD device is significant to the discharge parameters. The capacitance is varied by varying the air gap width only as the dielectric sheet thickness is fixed. In this project, the air gaps in use were from 0.5mm to 3.0mm. The effective capacitances across the electrodes calculated from the dimensions of the electrodes arrangement are shown in Table 3.1. The capacitance of the dielectric layer is calculated from the formula, $C_{dielectric} = \frac{\varepsilon_r \varepsilon_0 A}{d}$, A being the area of the electrode, d the thickness of the dielectric layer. For the air-gap, its capacitance is calculated from $C_{air-gap} = \frac{\varepsilon_0 A}{x}$, x being the air-gap width. $C_{\text{dielectric}}$ is in series with $C_{\text{air-gap}}$ (Figure 3.7).

Table 3.1: Calculated capacitances across the electrodes with **Glass** sheet as the dielectric barrier. Edge-effects are neglected.

Dielectric material	Glass				
Air-gap width / mm	0.5	1.0	1.5	2.0	3.0
Air-gap capacitance / pF	20.1	10.0	6.7	5.0	3.3
Dielectric-gap capacitance / pF	37.7				
Effective capacitance / pF	13.1	7.9	5.7	4.4	3.0

Table 3.2: Calculated capacitances across the electrodes with **Alumina** sheet as the dielectric barrier. Edge-effects are neglected.

Dielectric material	Alumina				
Air-gap width / mm	0.5	1.0	1.5	2.0	3.0
Air-gap capacitance / pF	20.1	10.0	6.7	5.0	3.3
Dielectric-gap capacitance / pF	90.4				
Effective capacitance / pF	16.4	9.0	6.2	4.8	3.2

3.1.4 Power Supply

A high voltage (H.V.) power supply was built to power the DBD. The power supply is voltage- and frequency-adjustable and can produce up to 25kV peak to peak voltage. The frequency of the high voltage output can be varied from 100s Hz to 12kHz.

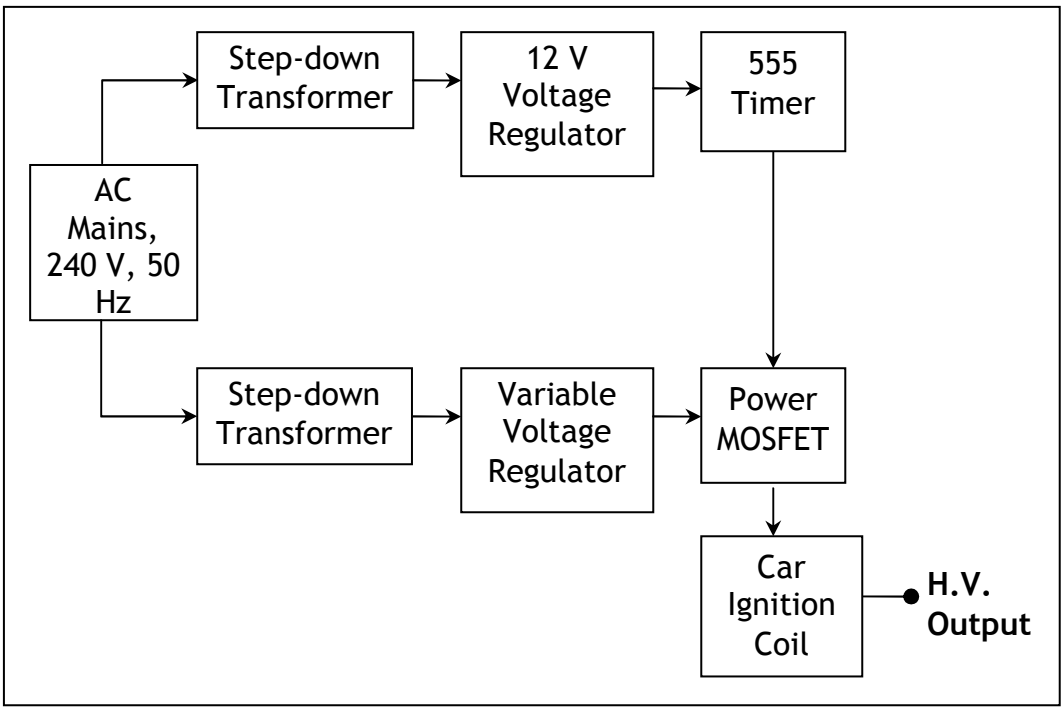

Figure 3.2: Schematic diagram of the high voltage generator circuit.

The schematic diagram of the high voltage power generator circuit is shown in Figure 3.2 while the detailed circuit is in Figure 3.3. It consists of five main parts: step down transformers, voltage regulators, 555 timer, power MOSFET, and a H.V. transformer (car ignition coil). The AC line voltage of $240V_{rms}$ is stepped down to 15V (12VA) and 15V (36VA). The 15V (12VA) output is supplied to a fixed voltage regulator (7812 IC) to drive the 555 timer, and the higher current rated 15V (36VA) output is applied to the variable voltage regulator to supply the bias voltage V_{DD} to the drain terminal of the MOSFET.

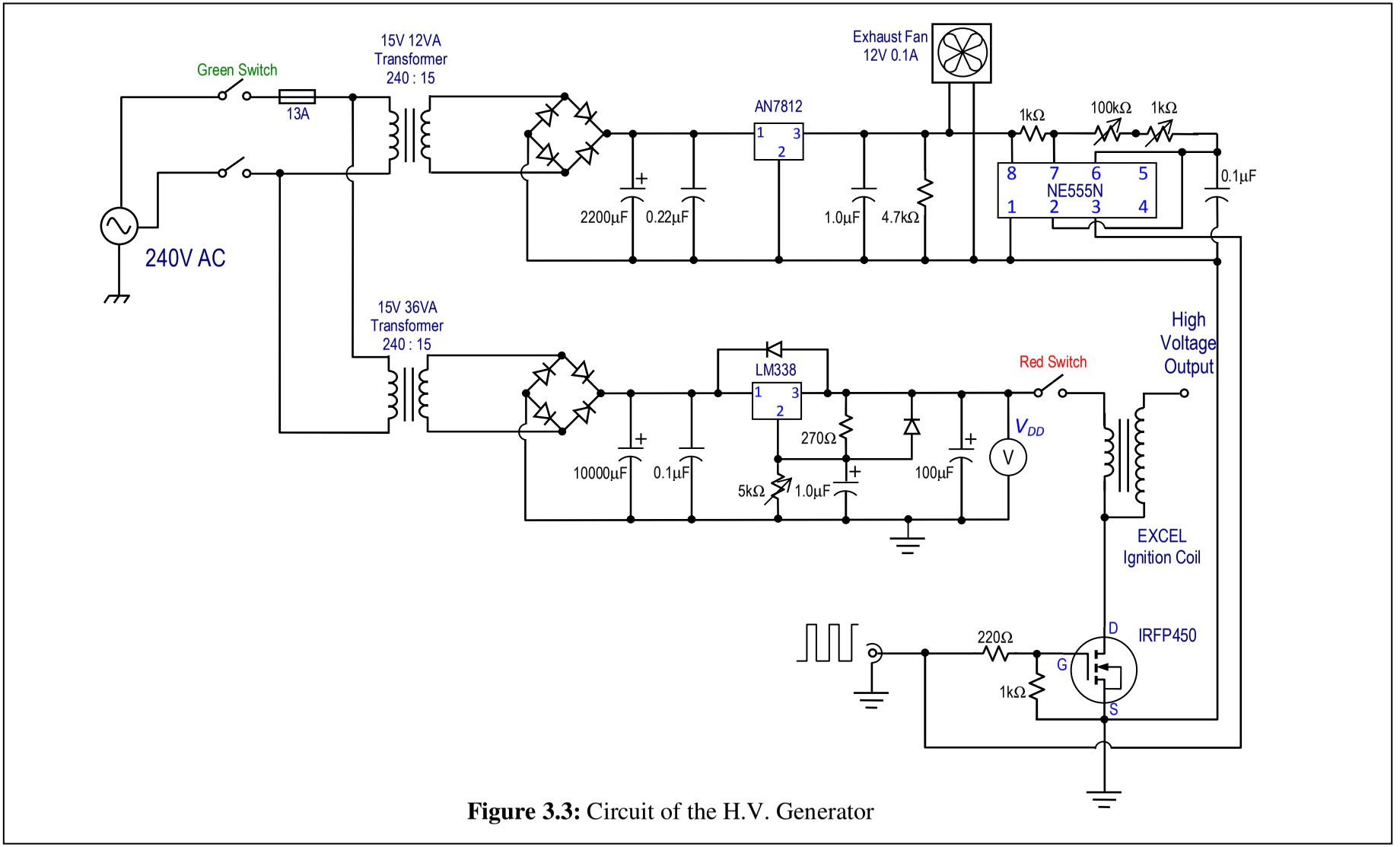

Figure 3.3: Circuit of the H.V. Generator

The 555 timer output controls the gate voltage of the MOSFET, with square pulses of adjustable duty cycle and frequency from the 555 timer. Bias voltage V_{DD} to the drain terminal of the MOSFET supplied by the variable voltage regulator controls the amplitude of the voltage output from the MOSFET. The MOSFET output is then amplified by the car ignition coil to generate the H.V. output. In short, the amplitude of the H.V. output of the power supply is controlled by the variable voltage regulator and its frequency is set by the 555 timer.

The power supply produces unipolar pulses at lower frequency (from 100s Hz to 2.5kHz) and sinusoidal waveform at higher frequency (from 6.5kHz to 12kHz). The mid-frequency range (2.5 kHz to 6.5 kHz) corresponds to the transition from unipolar to sinusoidal waveforms.

3.1.4.1 Output characteristics of the power supply

When the H.V. terminals are connected to the electrodes and the frequency (duty cycle is an important factor as well) is varied, the H.V. output waveform undergoes changes in terms of shape as well as amplitude. With glass dielectric layer, air gap distance of 3mm (no breakdown in the gap over a large range of frequency), and V_{DD} kept at 16.0V; the H.V. output is in the form of almost unipolar pulses (Figure 3.4) within the frequency range of 100Hz to 2.5kHz. The voltage amplitude (peak to peak) increases as frequency is lowered from 2.5 kHz. The “unipolar” pulses exhibit a distinct positive H.V. pulse (induced at the falling edge of the 555 timer square pulse) with some ripples at the tail-end. No damping attempt was made to eliminate this ripple. The magnitude of the largest negative swing of the ripple is about 20% of the H.V. positive pulse. It is noted that the duty cycle is close to 50% or the mark-to-space ratio is close to 1.

In the frequency range of 2.5kHz to 6.5kHz (duty cycle and mark-to-space ratio are also increased), the main positive pulse reduces while another peak in-between successive positive pulses grows (Figure 3.5(a)). At the same time, the negative peaks also grow (Figure 3.5(b)). The peak to peak amplitude of the voltage is low compared to the unipolar pulses and the sinusoidal wave. At the larger frequency end, two positive peaks merge and a sinusoidal signal begins to take shape (Figure 3.5(c)), while the amplitude starts to increase.

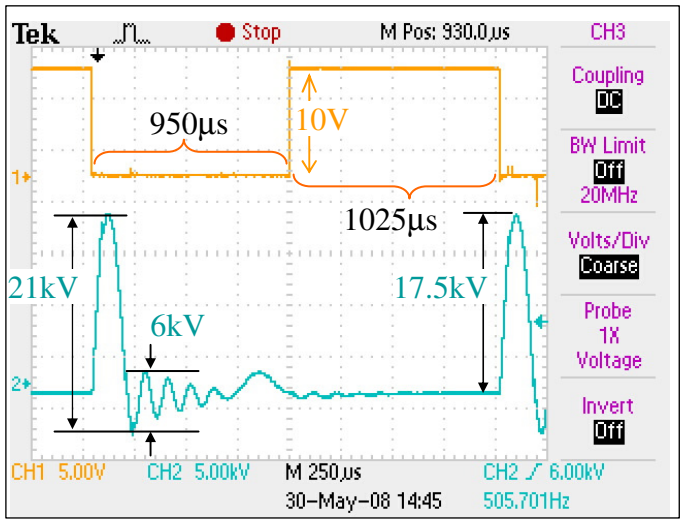

Figure 3.4: Waveform of "unipolar" voltage pulses (lower signal) with a distinctive main pulse followed by some ripples obtained at 500Hz with glass as dielectric, air gap of 3mm, and V_{DD} at 16.0V. The upper signal is the 555 timer output voltage.

Distinct sinusoidal output waveform is recorded for frequencies of 6.5 kHz and above (corresponding to duty cycle close to 80% and higher and mark-to-space ratio of 4 and higher). The voltage amplitude peaks at the resonance frequency defined by the configuration of the DBD system.

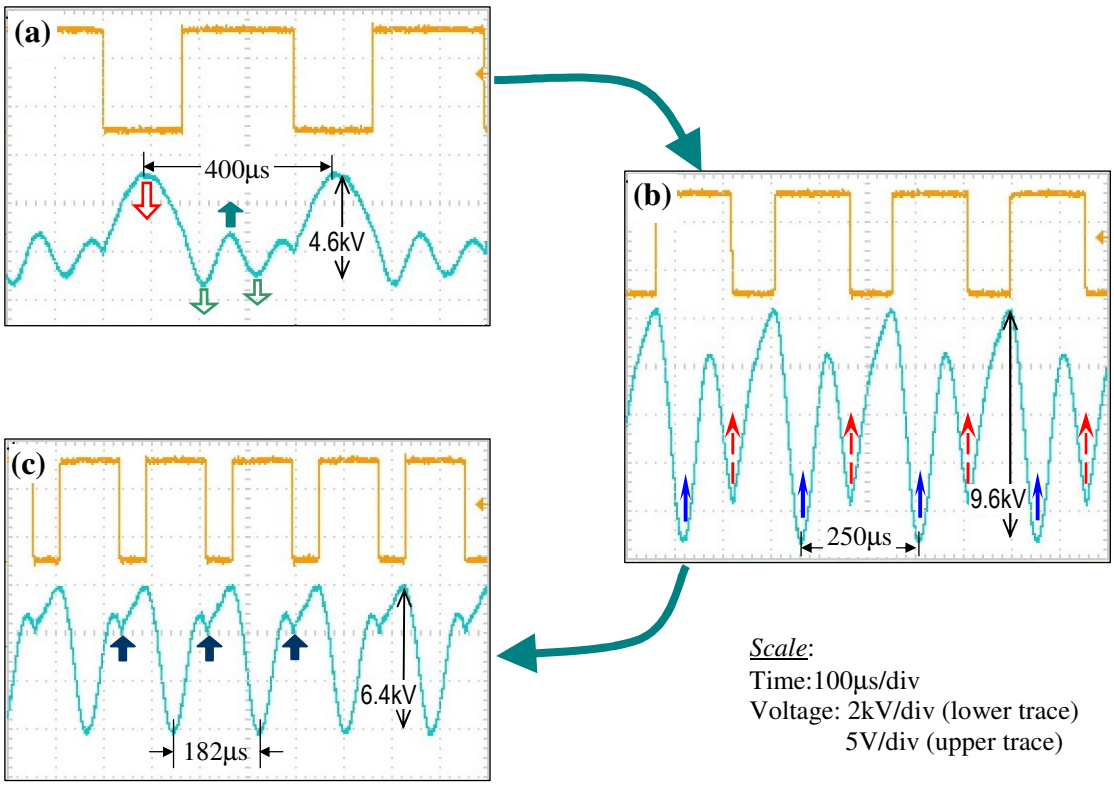

Figure 3.5: Waveform of the output voltage changes in shape and amplitude as frequency is increased from (a) 2.5kHz to (b) 4kHz to (c) 5.5kHz (in DBD with glass dielectric, air gap of 3mm, and V_{DD} at 16.0V). Duty cycle and (mark-to-space ratio) in (a), (b) and (c) are 59% (1.4), 64% (1.8) and 67% (2.0) respectively.

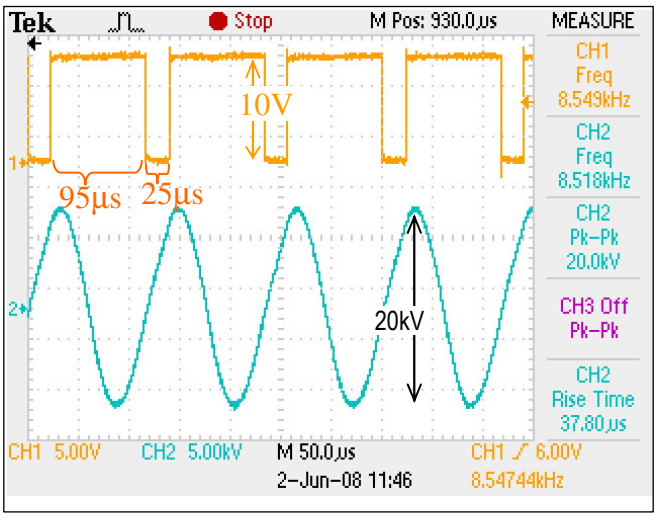

Figure 3.6: Distinctive sinusoidal waveform is obtained at frequencies 6.5kHz and above (in DBD with glass dielectric, air gap of 3mm, and V_{DD} at 16.0V)

3.1.5 Circuit Equivalent Schematic diagram of DBD

The equivalent circuit of the DBD discharge gap may be represented as shown in Figure 3.7 (Panousis, 2009). The gap between the pair of parallel plate electrodes is represented by two capacitors in series, one due to the dielectric layer, $C_{\text{dielectric}}$, the other contributed by the air gap, C_{gap}, when there is no discharge. A resistive load, $Z_{\text{discharge}}$, is paired in parallel with the air-gap capacitance if the discharge is ON, draining more current to the discharge gap. The DBD is essentially a capacitively coupled discharge.

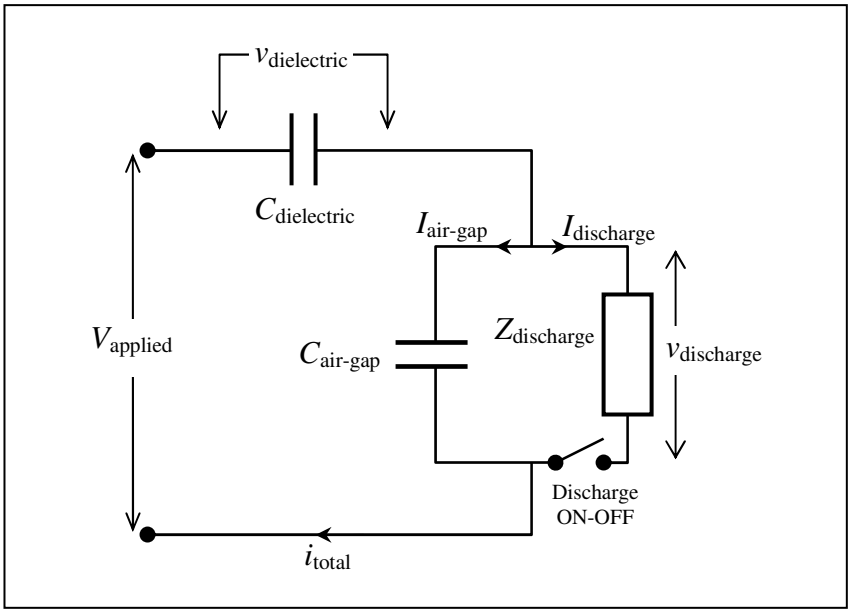

Figure 3.7: Equivalent circuit scheme of the DBD dielectric layer and discharge gap.

3.2 Data Acquisition and Measurement Tools

3.2.1 High Voltage Probe

The DBD discharge voltage was monitored via a Tektronix P6015A high voltage probe. The probe has silicon filling dielectric high and measures high voltages with 1000X attenuation. It is capable of measuring DC voltage up to 20kV or AC 40kV

(peak to peak) with pulse width lesser than 100ms. Bandwidth of the probe is 75MHz which is higher than frequency used for the discharge. It has compensation range of 7-49 pF with a small compensation box fitted before input to the oscilloscope.

3.2.2 Current Probe

The discharge current was measured by a Pearson current probe Model 4100 (hole diameter 0.5 inch). It has sensitivity of 1V/A. The probe is capable of handling maximum peak current of 500A and rms current of 5A with usable rise time of 20ns. The 3dB point of its frequency response is at 140Hz (low) and 35MHz (high).

3.2.3 Oscilloscope

The Tektronix TDS 2024B digital oscilloscope with bandwidth of 200MHz and 2GS/s was used for data acquisition. The input impedance of the oscilloscope is 1MΩ in parallel with 20pF. Data and signal attained by the oscilloscope can be saved to a removable mass storage (flash drive) through a USB port.

3.2.4 Digital Camera

The physical appearance of the discharge is captured using Canon EOS 40D digital SLR camera. It features 10.1 million effective pixels and has variable shutter speed of 0.125ms (1/8000) to 30s.

3.3 Optical Emission Measurement Setup

3.3.1 Spectrometer Setup

The optical emission from the DBD plasma was detected via the Ocean Optics HR4000 Spectrometer. The HR4000 is responsive in the spectral range of 200-662nm.

The spectrometer has an electronic shutter to control the amount of photon collected and prevents saturation. The function of the spectrometer is similar to a camera shutter which enables signal integration time ranging from 3.8ms to 10s. Optical resolution of the spectrometer is dependent on the grating (H2 grating with groove density 600 lines/mm blazed at 400nm) and slit (10 microns) installed. It has best efficiency (>30%) within the range of 230-750nm (Figure A.1 in Appendix) with spectral range of 425-445nm. The detector is a Toshiba TCD1304AP CCD linear CCD array with 3648 detector elements.

The light emission is brought to the slit of the spectrometer via a 2m length 0.4mm diameter fiber optic conduit. Its transmission curve is given in Figure A.2 in Appendix. In the optical emission measurement of the DBD plasma, the tip of the fibre optic conduit is placed 1.9cm away from the edge of the electrode as shown in the Figure 3.8.

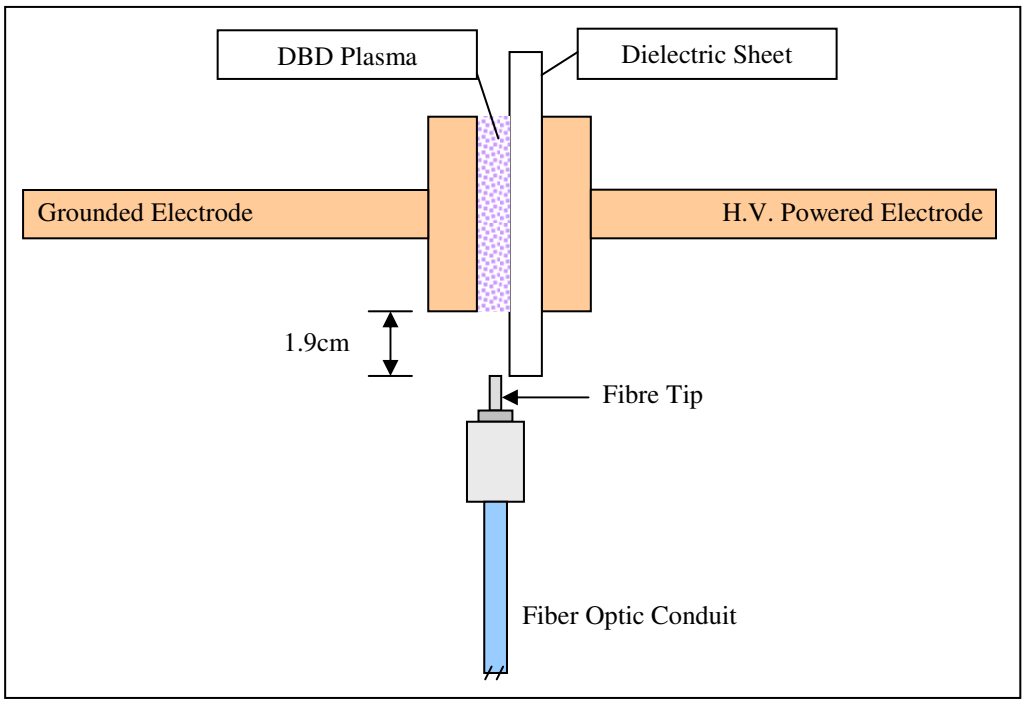

Figure 3.8: Position of fiber optic tip.

3.3.2 Calibration of the Spectrometer

The spectrometer was wavelength calibrated in the factory before it was delivered for use in this experiment. To ensure the accuracy of the spectrometer, a simple calibration was done using mercury cadmium spectral lamp in the laboratory. The visible spectral lines from the lamp are listed in the table below.

Table 3.3: Emission lines of mercury - cadmium lamp.

Element	Spectral Line (Å)
Hg	6234.4
	5769.6
	5460.7
	4358.4
	4046.6
Cd	5085.8
	4678.2

Table 3.4: Observed emission lines by the HR4000 Spectrometer

Element	Spectral Line (Å)	Observed from HR4000 (Å)	Δ(Å)
Hg	6234.4	-	-
	5769.6	-	-
	5460.7	5459.9	−0.8
	4358.4	4357.2	−1.2
	4046.6	4044.2	−2.4
Cd	5085.8	5084.2	−1.6
	4678.2	4676.1	−2.1

The observed emission lines were always shorter than the standard lines by ≤ 0.24nm (2.4 Å) within the range of 405-546nm.

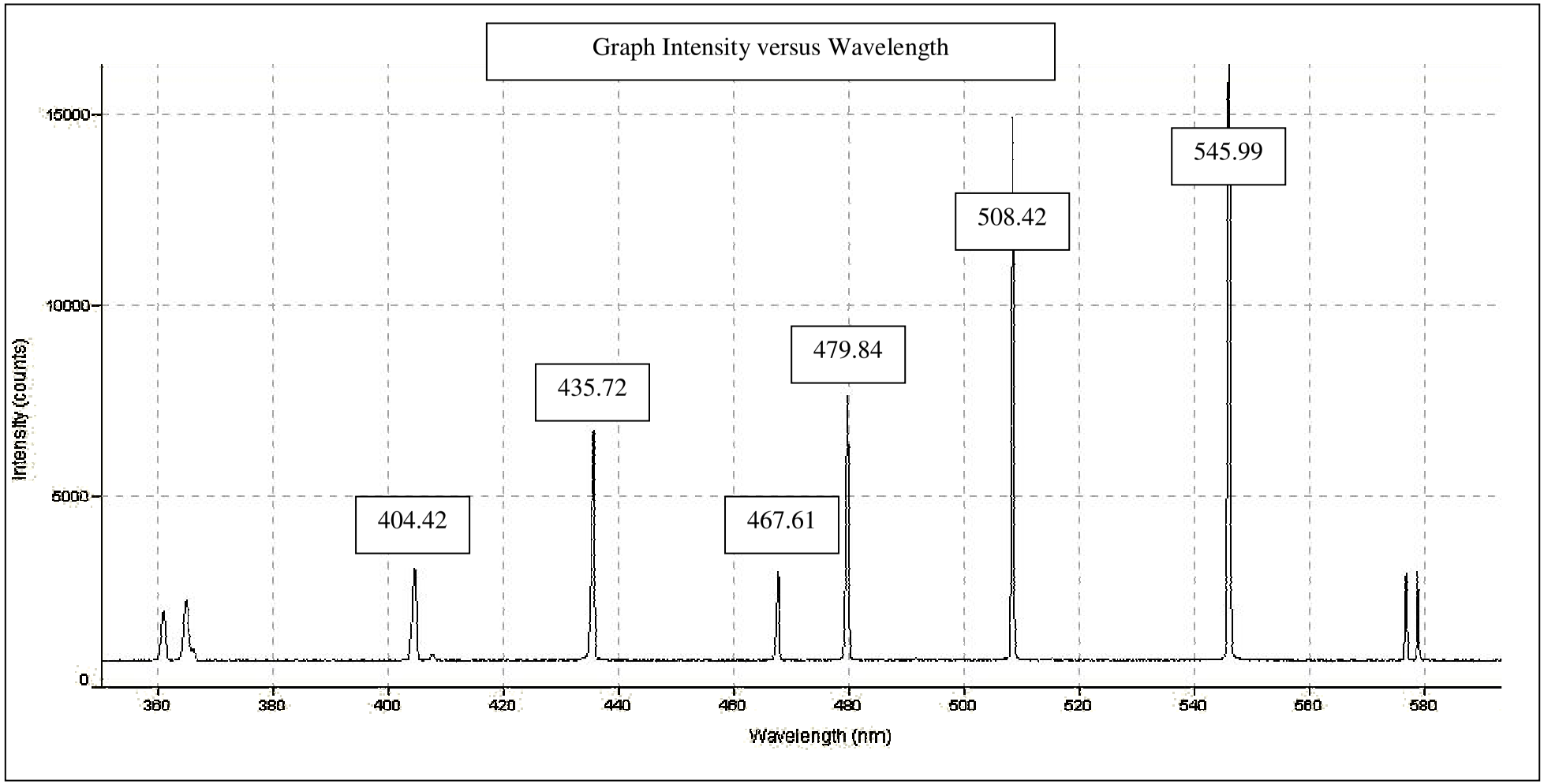

Figure 3.9:
Spectral lines observed from *Ocean Optics* HR4000 spectrometer with Hg – Cd spectral lamp as light source for wavelength calibration purpose.

The optical resolution (in nm FWHM) of the HR4000 Spectrometer is estimated as follows:

Table 3.5: Specification of the HR4000 spectrometer and slit installed.

Spectral range	Number of Detector Elements	Slit Installed	Pixel Resolution with Slit Installed
425-445nm	3648	10 microns	~3.7 pixels

(i) Dispersion (nm/pixel) = Spectral Range of the Grating/Number of Detector Elements

= 445/3648

= 0.122 nm/pixel

(ii) Optical Resolution (in nm) = Dispersion × Pixel Resolution

=0.122 nm/pixel × 3.7 pixels

= 0.5 nm = 5Å (FWHM)

The optical resolution of the spectrometer is approximately 0.5nm (5Å). This is twice the deviation error between the standard and observed emission line wavelength. Hence, it can reasonably be assume the spectrometer is accurate and calibrated.

3.4 Bacteria Inactivation

3.4.1 Disinfection Target

Three bacterial species were selected as the disinfection target for this project. These bacteria were selected based on their economic importance as these bacterial pathogens are common causes of foodborne infection in Malaysia. One of the goals of this project was to develop a system to inactivate bacteria so that the system can be adapted as a sterilization unit.

3.4.1.1 ***Escherichia coli ATCC 25922***

Escherichia coli (***E. coli)*** is a gram-negative bacterium that is commonly found in the digestive system of animals, food, and waste water. These non spore-forming rod bacteria typically measure 0.8µm wide and 2µm long (Sundararaj *et al.*, 2004). Most of the *E. coli* strains are harmless. However, some of the strains can cause serious food poisoning and illness.

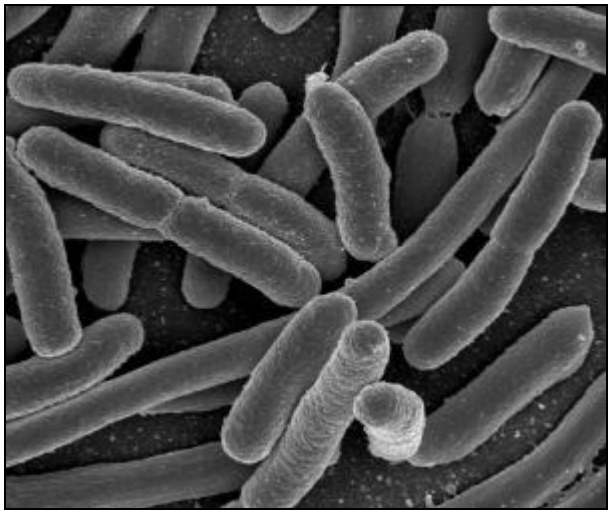

Figure 3.10: Scanning Electron Micrograph of *Escherichia coli*. (UCLA, 2008)
(Credit: Rocky Mountain Laboratories, NIAID, NIH)

3.4.1.2 *Salmonella enteritidis*

Salmonella enteritidis is a gram-negative, non spore-forming bacterium which is rod shaped and usually measures 0.7 to 1.5µm in diameter, and 2 to 5µm in length. They are normally found in animals, reptiles, humans and polluted water. Many food-borne illness are caused by *Salmonella*, particularly *Salmonella enteritidis* can cause serious diarrhoea. Millions of people around the world are infected by *Salmonella enteritidis* each year through consumption of contaminated eggs. .

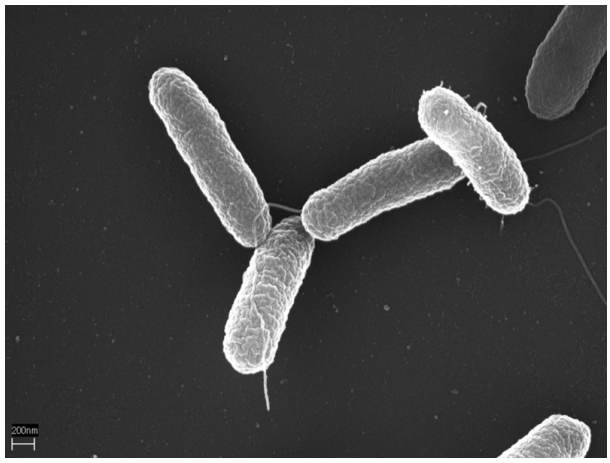

Figure 3.11: *Salmonella enteritidis* (Korbel, 2005)
(Credit: Volker Brinkmann, Max Planck Institute for Infection Biology, Berlin, Germany)

3.4.1.3 *Bacillus cereus*

Bacillus cereus is a gram-positive, rod-shaped bacterium. As a member of the genus *Bacillus*, it can produce protective endospores which enable them to survive in harsh environment. The spores of *Bacillus* are found to be resistant to heat, radiation, and disinfectants. Some of the strains are harmful to humans while others can be beneficial as probiotics for animals (Ryan and Ray, 2004). These bacteria usually

measure 1µm wide, 5-10µm long and they reproduce in short chains. The *Bacillus* species are a frequent cause of contamination in medical material and food product.

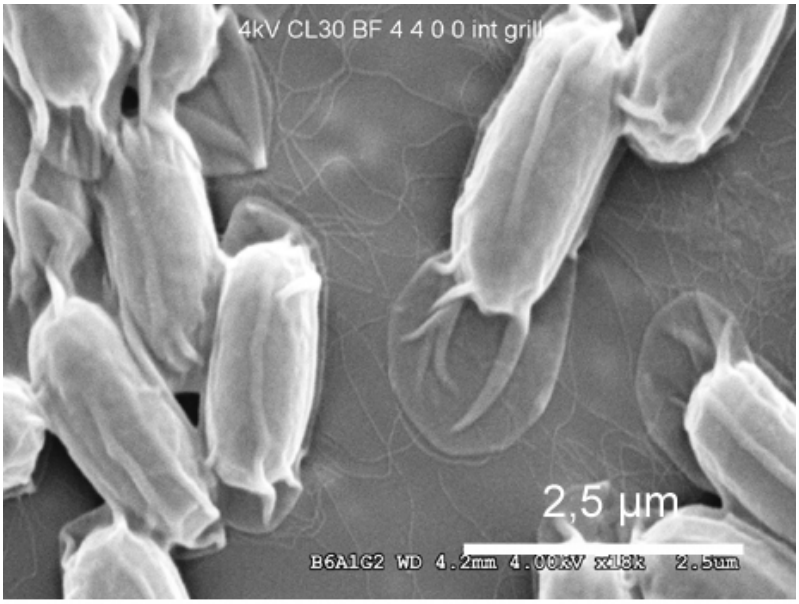

Figure 3.12: *Bacillus cereus*. (Inra Lille, 2010)

3.4.2 Preparation of the Bacterial Cultures

The bacteria used for sterilization purpose need to be prepared in a proper way, otherwise contamination can render the results useless. The preparation of the agar plates, and bacterial cultures were carried out aseptically in the Microbiology Laboratory, Institute of Biological Studies, Faculty of Science, University of Malaya. Plating of bacterial cultures was done in the Biosafety Cabinet Class II.

3.4.2.1 Preparation of the agar plates

The agar plates prepared for this study were the Nutrient agar and *Luria Bertani* (LB) agar. For the Nutrient agar, an amount of 5.2g of nutrient broth powder was weighed and then transferred into a 500ml empty cleaned glass bottle. Distilled water

was then added to fill the glass bottle up to the 400ml mark. The bottle was shaken to dissolve the powder. For the LB agar, 4g of tryptone, 2g of yeast extracts, 2g of Sodium chloride, and 6g of Bacteriological agar powder were used instead. The rest of the preparation was similar to that for Nutrient agar. The media were then sterilized by autoclaving at 121°C for 20 min.

After autoclaving, the solution was cooled to about 50°C and then poured into sterile petri dishes. The media was allowed to set for about 30 min in a laminar flow cabinet. The agar plates were then air-dried in the cabinet under UV illumination for about 30 minutes before being used.

3.4.2.2 Preparation of bacterial cultures

The bacterial cultures to be used must be a pure culture. The bacteria strain, for instant *E. coli ATCC 25922* was obtained from the glycerol stock that was stored at minus 20°C. By using an inoculation loop, a loopful of inoculum was transferred to the freshly prepared agar medium. A technique called the four-way streaking was applied to obtain well isolated colonies of bacteria. The loop was streaked from the initial corner to another corner across the plate in lines (Figure 3.13). A second streak was applied across the end of these lines to yet another corner. A third streak was similarly applied. The final and fourth streak was a single line ending with a random 'S' line as shown in Figure 3.13. The inoculation loop was sterilized by placing it in the flame of a Bunsen burner in-between each successive streak.

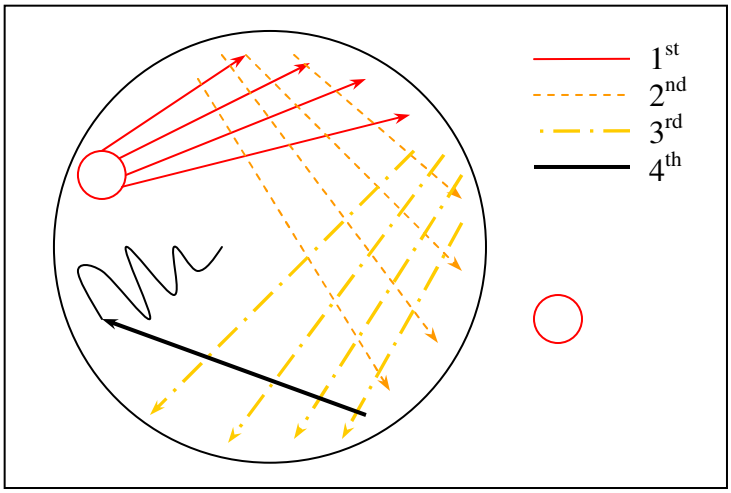

Figure 3.13: Series of streaks in the streaking technique.

After the streaking process, the agar plate was placed in an incubator at 37°C for 24 hours. An example of the appearance of the agar plates after incubation is shown in Figure 3.14. The single colony (appears as a single visible spot) of bacteria appearing on the agar plate originates from a single cell bacterium, isolated via the streaking process. The single colony of the bacteria consists of millions of bacteria being regenerated of the single cell bacterium. Eventually, all the bacteria in this particular colony are of pure strain and contain only one species of bacteria.

All three species of bacteria were prepared using the same streaking method described above. However, *Escherichia coli ATCC 25922* and *Salmonella enteritidis* are colony- forming while *Bacillus cereus* is a web forming type. Hence, for *Bacillus cereus*, the pure strain bacteria were observed as a single web structure.

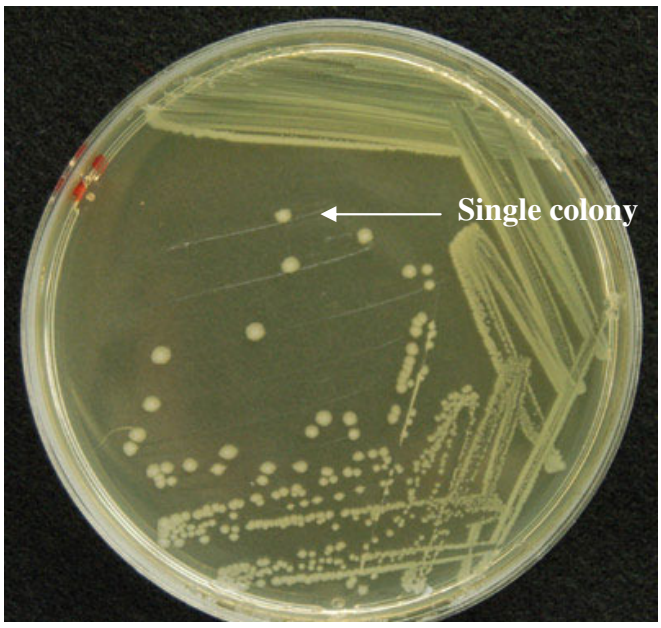

Figure 3.14: Single colony bacteria observed as a visible spot after incubation. (Liao and MacWilliams, 2006)

3.4.2.3 Incubating/Growing the bacteria

After obtaining the pure bacteria strain, the bacteria needs to be grown in nutrient rich broth overnight. Two microfuge 1.5ml tubes were used with one labeled 'bacteria' and the other 'Control'. 1.0ml of LB broth was pipetted into each tube. A small amount of bacteria was scooped up from the single colony of bacteria in the agar plate using a pre-sterilized inoculating loop and then stirred into the tube labeled 'bacteria'. The two tubes were placed in a water bath shaker at controlled temperature of 37^{o}C for 24 hours. After this, the bacteria are finally ready for use. However, immediately before the sterilization treatment by the DBD system, the bacterial cultures were diluted first through the serial dilution procedure (described in the Section 3.4.3.1).

3.4.3 Sterilization via Plasma Treatment Procedure

For the investigation of effectiveness of DBD plasma to inactivate bacteria, the prepared bacteria were placed on the surface of the dielectric barrier for exposure to the plasma. The number of bacteria that survive after the plasma treatment were enumerated and analyzed. For this purpose, each treated sample will be transferred to a fresh agar plate. After 24 hours of incubation, if none of the bacteria survive the plasma treatment, the agar plate will show no sign of living bacteria at all. If some bacteria survive the plasma treatment, after the 24 hour incubation period, each bacterium will have grown and regenerated to become a colony of bacteria, appearing as a spot on the surface of the agar medium as shown in Figure 3.15.

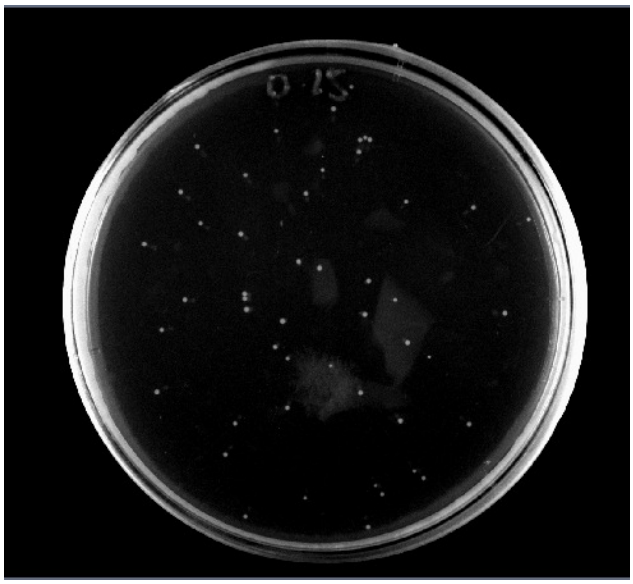

Figure 3.15: Each spot indicates a bacteria colony regenerated from one bacterium cell.

3.4.3.1 Serial dilution procedure

For accurate estimation of the bacteria grown in a petri dish, the upper limit is set to 300 bacteria colonies per petri dish as a standard practice. Bacteria solution of high density (exceeding 300 bacteria in number) is considered too numerous to count "TNTC". Thus the bacteria solution prepared in the microfuge tube needs to be diluted to a suitable level before usage. For this procedure, 8 microfuge tubes were prepared and labeled from "-1" to "-8", each number representing the power of dilution of the solution in the tube. Then 900µl of 0.85% saline water was pipetted into each sterile tube. From the tube labeled 'bacteria' of the earlier prepared pure-strain bacteria in LB broth (Section 3.4.2.3), 100µl of the bacteria solution was pipetted and injected into tube labeled "-1" followed by a mixing action. "-1" means the bacteria solution was diluted 10^1 times or 0.1 of the original bacteria density.

For the next dilution, 100µl was extracted from tube "-1" and injected into tube "-2", the bacteria is now 0.01 or to the power of 10^{-2} of the original density. With a repeated sequence of the procedure, the 8^{th} tube (labeled -8) of bacteria solution will have a dilution to the power of −8.

3.4.3.2 Bacteria sterilization/ Inactivation

The bacteria solution diluted in the previous procedure is now ready for bacteria inactivation treatment in the DBD. A control sample was required for the indication of initial bacteria count whereby it will undergo no plasma treatment. If the treatment experiments are carried out with bacteria of dilution "-5" then the control sample is prepared from the same tube of "-5" bacteria density. In most cases, another control sample of higher dilution of, say, "-8" is prepared as a precaution, in case the control "-5" is found to be unusable or "TNTC".

Sterilization treatment was carried out with a 50µl of the bacterial suspension from the "-5" dilution placed on the glass dielectric barrier. The sample was then exposed to the DBD plasma for a pre-determined time duration ranging from 5 seconds to 4 minutes. The treated bacteria suspension was transferred to the agar plate by washing it down with 100µl or more saline solution. A "hockey stick" shaped glass rod was used to smear the bacteria solution evenly over the agar surface. The "hockey stick" has to be sterilized with alcohol wipe and flame every time before each smearing process to avoid cross contamination. The apparatus for the experiment such as the glass dielectric barrier and glove were cleaned with 70% alcohol each time before usage to ensure that it is bacteria free and to prevent cross contamination.

The treated samples and controls were incubated for 24 hours at 37 ^{o}C. The number of colonies formed after incubation was counted and survival rate of the bacteria was analyzed.