

THE EFFECTS OF ELECTRIC FIELD ON MICROORGANISMS IN SEMI-LIQUID AND LIQUID EDIBLE FOOD SUBSTANCES

In partial fulfillment of the requirements

for the degree of

Bachelor of Science

in Electrical and Electronic Engineering



Submitted By

ADEL BASHER RAHMAN

Student ID: 10321045

MOHD. WASIM CHOUDHURY

Student ID: 10121063

AUGUST 2015

DECLARATION

We hereby declare that the thesis titled "**The Effects Of Electric Field On Microorganisms In Semi-Liquid And Liquid Edible Food Substances**", submitted to the Department of Electrical and Electronic Engineering, BRAC University for the fulfillment of degree in Bachelors of Science in Electronics and Electronic Engineering, is our original work. Any information used from other sources has been acknowledged in the reference section.

Submitted by:

Adel Basher Rahman

Student ID: 10321045

Mohd. Wasim Choudhury

Student ID: 10121063

Signature of the Supervisor

Ms. Marzia Alam,

Department of Electrical and Electronic Engineering,

Brac University

ACKNOWLEDGEMENT

Over the course of this thesis, for the past year, we have devoted ourselves to this work, the progression of which have made would never have been culminated on our own and for that; we have found ourselves indebted to many members of the faculty.

First and foremost, we are sincerely greatly to our supervisor, Ms. Marzia Alam, the senior lecturer of EEE department, BRAC University for whose without continuous positive support, guidance and counsel for the completion of this work would not have been otherwise possible.

We would also like to show our gratitude to Professor Mahbubur Rahman, of MNS department, BRAC University, whose expertise in the area of microbiology has helped us manifold and has given invaluable assistance, whenever required. We are also indebted to Ms. Asma the laboratory technical officer of microbiology lab, MNS department, whose dedication, practical support and patience for the thesis made it possible for us to complete our thesis. Lastly, we would like to thank all the other lecturers, faculty members and other technical offers of the BRAC University, for their involvement in the completion of our thesis.

ABSTRACT

Pulsed electric field (PEF) is a new, emerging and a non thermal method of processing of food preservation technique, which uses pulsating electric field for the inactivation of microorganisms. The process causes a minimal effect on food quality characteristics such as smell, taste, color and texture. PEF can be especially used for processing liquid and semi-liquid edible food substances. Studies conducted on the energy requirements have concluded that PEF is an energy efficient process compared to the traditional thermal methods such as pasteurization.

In this study the PEF was applied to various edible food items: raw milk, fruit juice, sugarcane extract and tap water. High energy electric field, ranging from 400 V/cm up to 1600 V/cm of frequency 50 Hz was applied and the corresponding microbial colony growth was counted. Here effectiveness of the AC field against the DC field was also compared; the impact of number of pulses applied and effect of thickness of the metal plates on the growth of the microorganisms were studied. The experimental results are encouraging and are supportive of the applied technique.

TABLE OF CONTENTS

CHAPTER 1: INTRODUCTION	1
1.1 Historical Background	4
1.2 Motivation	5
CHAPTER 2: RESEARCH METHODOLOGY	6
2.1 The Effect on the Microbial Growth for Various Samples Exposed under Variable AC Fields ranging from 700v/cm up to 1600 v/cm.	8
2.2 The Effect of Number of Pulses on the Microbial Growth	8
2.3 A Comparison between the AC and DC Field	8
2.4 The Effect of Thickness of the Plates	9
2.5 Electroporation of Cell Membrane due to PEF	9
2.6 Derivation of $E = v/d$	12
CHAPTER 3: EXPERIMENTAL SETUP	14
3.1.0 The Electrical System Setup and the Equipment Used	15
3.1.1 Testing the Rating of a Transformer	17
3.2.0 The Biological Systems	18
3.2.1 The Agar Gel Preparation	18
3.2.2 Preparation of EMB Agar	18
3.2.3 Preparation of Macconkey Agar	19
3.2.4 The Difference between EMB and Macconkey Agar	20
3.2.5 Safety Protocol	20
3.2.6 Preparation of Saline Solution	20

3.2.7 Preparation of Sample Using Serial Dilution Method	21
3.2.8 Procedure of Colony Count in Petri Dishes	22
CHAPTER 4: EXPERIMENTS AT DIFFERENT TESTING CONDITIONS	23
4.1.1 Experiment 1: Samples at Different AC Field Length	24
4.1.2 Equipments Used	24
4.1.3 Experimental Procedure	24
4.2.1 Experiment 2: Effect of Number of Pulses	26
4.2.2 Equipments Used	26
4.2.3 Experimental Procedure	26
4.3.1 Experiment 3: Comparing AC with DC Field	28
4.3.2 Equipments Used	28
4.3.3 Experimental Procedure	28
4.4.1 Experiment 4: Effect of thickness of the plates	30
4.4.2 Equipments Used	30
4.4.3 Experimental Procedure	30
4.5.1 Safety Protocols	33
4.5.2 While Performing the Experiments with the Electrical Equipments in the Power Laboratory	33
4.5.3 While performing the Experiments in the Biology Laboratory	33
CHAPTER 5: DATA AND RESULTS	34

5.1.0 Experimental Conditions	35
5.1.1 Sample Data at Different AC field Length Showing the Colony Count	35
5.1.2 Sample Data at Different ac Field Length Showing the Microbial Count	35
5.2.0 Experimental Conditions	36
5.2.1 Sample Data showing the Effect of Number of Pulses on the Colony and the Microbial count	36
5.3.0 Experimental Conditions	37
5.3.1 Sample Data Showing the Effects of AC Field and DC Field on the Colony and the Microbial Count	37
5.4.0 Experimental Conditions	38
5.4.1 Sample Data Showing the Effect of the Thickness of the Plates to Plate Separation Distance to the Colony and the Microbial Count	38
CHAPTER 6: ANALYSIS AND DISCUSSION	39
6.1.0 The Effect on the Microbial Growth for Samples Exposed under Variable AC Fields	40
6.1.1 Relationship between the Microorganisms Survival Ratio as a Function of the Effective Applied Electric Field	44
6.1.2 Extrapolations of the Trend Lines and Interpretations	47
6.2.0 Effect of Number of Pulses	48
6.2.1 Graphical Analysis	50

6.3 AC Field Compared with the DC Field	53
---	----

6.4.0 Data Showing the relationship between of the Thickness of the Plates and Plate Separation Distance to the Colony and the Microbial Count	54
--	----

CHAPTER 7: DESIGNING AND MODELLING OF THE TEST CHAMBERS	56
--	-----------

7.1 Design of the Test Chamber	57
--------------------------------	----

7.2. Practical Static Chamber, as Constructed for the Experiment	59
--	----

CHAPTER 8: CONCLUSION AND FUTURE WORKS	61
---	-----------

REFERENCES	63
-------------------	-----------

APPENDIX	65
-----------------	-----------

LIST OF FIGURES

Figure [2.5.a]: Electroporation Affecting a Living Cell	10
---	----

Figure [2.5.b]: Electroporation Affecting a Living Cell, Step by Step	11
---	----

Figure [3.1.a]: High Voltage Variable AC Supply Unit 0-900v, 50Hz	15
---	----

Figure [3.1.b]: Testing of the Fixed AC Voltage Supply Unit (400v highest)	16
--	----

Figure [3.1.c]: Testing of the Variable AC Voltage Supply Unit (0~800v)	16
---	----

Figure [4.1.a]: Testing and Placing of the Glass Slide Position between Two Plates	25
Figure [4.1.b]: Removing the Glass Slide, Placing the Sample and Notice the Separation Distance	25
Figure [4.1.c]: Glass Slide with Sample, Placed into position and the Power is Switched on	25
Figure [4.2.a]: Equipments: Plate Holder, Durham Tubes, Vials, Pipette Tips, Stopwatch	27
Figure [4.2.b]: The Setup is Connected to the Supply, Durham Tubes Filled with Samples are Placed in the Parallel Plate Holder	27
Figure [4.3.a]: The Setup is Very Similar to Experiment 4.1.1, However Once it is Connected to the Variable DC Supply and the Other Time to a Variable AC Supply	29
Figure [4.4.a]: The Equipments and the General Setup	31
Figure [4.4.b]: The Experiment with one Single Plate (1mm Thickness)	31
Figure [4.4.c]: Increasing Thickness of the Plates to 16mm	32
Figure [4.4.d]: Placing the Sample on the Glass Slide and Connecting the 16mm Plates to Power Supply	32
Figure [4.4.e]: The Top View of the Figure 4.4.C	32
Figure [7.1.a]: Prototype of the Two Chamber “Plates”	59
Figure [7.1.b]: Prototype of Static Chamber Used While Conducting Experiments	59
Figure [7.1.c]: The Horizontal View of the Prototype	60

Figure [7.1.d]: The Top View of the Prototype	60
---	----

LIST OF TABLES

Table [5.1.1]: Samples at Different AC Field Strength	35
Table [5.1.2]: Samples at Different AC Field Strength	35
Table [5.2.1]: Effect of Number of Pulses	36
Table [5.3.1]: AC Field versus DC Field	37
Table [5.4.1]: Thickness of the Plates	38
Table [6.1.1]: Samples at Different AC Field Strength	42
Table [6.2.1]: Effect of Number of Pulses	48
Table [6.3.1]: AC Field versus DC Field	53
Table [6.3.2]: Thickness of the Plates	54

CHAPTER 1: INTRODUCTION

People are increasingly becoming more interested in consuming processed food items, which have “natural” flavorings and colors, furthermore also at the same time, having the minimal amount of chemical: preservatives, coloring agents, artificial flavorings also having low salt and sugar content. They are also demanding processed food items, which have adequate shelf life for distribution and as well as a reasonable period of home storage before consumption. One of the traditional methods and popular technique of food preservation are by the thermal processing method i.e. by heating. The other modern methods of food preservation include: irradiation, ozone treatments, microwave heating, microfiltration, the application of high-voltage arc discharges and bombardment by ultraviolet radiation, and. The application of high electric field or pulsed electric field (PEF) is a new, innovative and energy saving method which is suitable especially in the cases of liquid and semi-solid edible food substances such as: milk and dairy products, fruit juices and extracts, soups, purees, sauces, alcoholic beverages and drinking water [1] [2] [3].

The PEF also provides an alternative to the thermal treatment of food substances and other edible liquid or semi-solid foods. The technique involves the application of high voltage electric pulses to food substances placed either in between two inert plates (the static chamber process) or two chemically inert electrodes inserted into a chamber as the liquid edible food substances flows through it (the continuous chamber process). The amount of heat produced is negligible as compared to, with other traditional methods, thus consequently this method not only preserves the freshness of the treated food items, but also aids in increasing the shelf life of the food [4]. Most liquid food items are generally good conductors of electricity as they have high concentrations of free ions, thus making them ideal for the PEF treatment. The PEF treatment inactivates the microorganisms by inducing electrical potential beyond the critical strength of the cell membrane, which in turn causes the charge separation in the membrane, which ultimately leads to cell rupture [5].

The PEF is of considerable interest to the food processing industry as a technique which can be adapted for processing foods on a very large scale and comparatively requiring less energy than the traditional, conventional, thermal methods: akin to the ohmic heating and other aforementioned electrical processes. Some of those methods require a continuous electrical current to be supplied throughout the food material in order to heat it evenly, for the entire duration of the treatment, however the PEF is not intended to heat the food items, but rather warrants the application of short pulses of high voltage to food items placed between two electrodes, which destroys unwanted microorganisms without causing any significant heat generation [6].

The fresh milk makes an excellent choice to test the effects of PEF because due to its composition. The milk provides an excellent medium for the growth of various external microorganisms which can change its properties: causing it to go sour, lipid hydrolysis and rancidity, the development of bitter flavors and destabilization of milk fat emulsion. The traditional method of preserving milk by pasteurization (a thermal process), although widely accepted and practiced, is not without having its disadvantages. The heat can modify milk components, for instance the decrement of the calcium ions, the denaturing of milk proteins and the degradation of vitamins and minerals. It also can affect the texture, quality and the taste of the milk [7] [8].

In this study, the PEF was applied to various samples of edible food items such as: raw milk, fruit juice, sugarcane extract and tap water. High energy electric field, ranging from 400 V/cm up to 1600 V/cm of frequency 50 Hz was applied and the corresponding microbial colony growth was counted. Here, effectiveness of the AC field against the DC field was also compared; the impact of number of pulses applied and effect of ratio, of thickness of the metal plates to the separation distance of the test chamber, on the growth of the microorganisms were also studied. The experimental results are encouraging and are supportive of the applied technique; however more experimentation and further analysis are needed to perfect the study.

1.1.0 HISTORICAL BACKGROUND

The lethal effect of electric current on microorganisms was already tested and documented before the end of the nineteenth century; however these effects were produced by either applying direct or low-frequency alternating current, resulted from thermal or electrochemical effects

In the 1920s, a new process called “Electropure” was introduced in the USA and Europe This was one of the first attempts to use electricity for milk pasteurization, it was carried out by the application 220 V alternating current within a carbon electrode treatment chamber. About 50 such plants were in operation until the 1950s, however due to the rising energy costs and competition with other thermal preservation technologies such as Ultra High Temperature (UHT); these (ohmic) heating plants were gradually phased out [9].

One of the major drawbacks of applying electricity directing into the food items were the lethal effects of electrochemical reactions such as the hydrolysis of chlorine which was found when subjecting food high voltages. The Pulsed discharges of high voltage electricity across two electrodes for microbial inactivation were first investigated in the 1950s resulting in a process called “electro-hydraulic” treatment. The later experiments conducted revealed that pulsed electric fields can be applied for disruption of cells in food material and were further developed and expanded to the inactivation of microorganisms and wastewater treatment [10].

Important early patents in the application of PEF for treatment were applied by Krupp, Germany, developing two new processes, for the inactivation of microorganisms in milk and fruit juices with electric field strength up to 30 kV/cm, however because to high heat produced due to high energy dissipation and consequently the high costs of operation inhibited successful industrial application. Later patents were applied by an American with electric fields in the range from 10 to 25kV/cm and the microbial inactivation and effect on fruit juice quality was investigated by Dunn and Pearlman showing an increase of shelf-life of about one week [11].

1.2.0 MOTIVATION

Today because of the advancement of science and technology life has become very smooth. Science has facilitated our way of life by discovering various wonders in our day to day life. There is hardly any field in which science has not worked abundantly. In case of purification of liquid food items like milk, juice etc science has discovered various ways out of which thermal or heating process is evident.

To make the life easier, another method which can be effective is the application of electric field in the liquid or semi solid food items. This is a non-thermal process which uses short pulses of electricity to obstruct the microbial growth in a particular food item and diminishes the detrimental effect. This is a process which is even safer as it safeguards the human body more by not allowing major changes in the properties of a specific food item.

Bangladesh is a developing country where the utilization of pulsed electric field can influence the country positively. As it is developing, the addition of this technology can take the country one step ahead. Today, the top food and beverage companies of Bangladesh like Aarong, Milk vita, Pran etc. are competing with one another to get the best possible response from the customers in the commercial market.

The objective of this method is to neutralize the microorganisms, hence to offer the customers better quality food. This technology is also very conducive as it maintains the original color, nutrition and the flavor of the food unlike the heating process. Furthermore, non-thermal methods lately have attained more response because of its high nutritional value. The application of electric field in the food items can be considered as one of the most promising methods for the purification of liquid food items as it can maintain the quality of the food items in reasonable costs [12].

CHAPTER 2: RESEARCH METHODOLOGY

The study and the area of research was primarily focused on the four different parameters which were: (a.) the effect on the microbial growth for various samples exposed under variable AC fields (b) the effect of number of pulses on the microbial growth (c.) a comparative study between effects of the AC and DC field and (d.) the effect of thickness of the plates. These are explained in further detail later on. The food samples of milk, fresh juice, tap water and sugarcane extract were prepared in 0.9% saline solution, having a neutral pH. All the experiments were conducted at room temperature.

To study the effect on the microbial growth for various samples exposed under variable AC field strength, the food samples were placed in a specially made glass chamber, having the plate separation of 0.5 cm and variable voltages were applied for a period of two minutes. To analyze the effect of number of pulses on the microbial growth, two parallel plates were used again having a plate separation of 0.5 cm. The samples were placed into Durham test tubes and each was removed after a certain time interval. To study difference between the AC and DC field, the sample were simultaneously placed in AC and DC fields for the same time interval. And lastly, to study effect of thickness of the plates, the thickness of the electric plates were varied for each step, whilst the separation distance between the chamber plates remained constant at 0.5 cm. The exposure time for each remained constant.

The schematic diagram of experimental setup is given in the Figure 3.1.a. The power supply used was custom made with a transformer which was able to produce 800V, 50 Hz, (although its highest rated voltage was 1.10 kV) from a line voltage of 220V, 50 Hz.

2.1 THE EFFECT ON THE MICROBIAL GROWTH FOR VARIOUS SAMPLES EXPOSED UNDER VARIABLE AC FIELDS RANGING FROM 700V/CM UP TO 1600 V/CM

Samples of different food substances were prepared, these were then exposed to variable AC fields for two minutes each and then the samples were collected, transferred and spread on to an agar growth medium. These were left for twenty-four hours in an incubation chamber and then the microbial colony growths were counted. One of each sample acted as control, to which no electric field was applied.

2.2 THE EFFECT OF NUMBER OF PULSES ON THE MICROBIAL GROWTH

The samples of milk were prepared, a set of five were exposed to an electric field of 800 V/cm and another set of five were exposed to an electric field of 1600 V/cm. After duration of 1, 2, 3, 5 and 10 minutes each sample was removed, collected, transferred and spread on to an agar growth medium. Since the supply voltage was 220V/ 50Hz, therefore the number of AC pulses was 3,000 pulses per minute and the pulses increased by this factor for every minute. These were left for twenty-four hours in an incubation chamber and then the microbial colony growths were counted. One sample of each acted as control, to which no electric field was applied.

2.3 A COMPARISON BETWEEN THE AC AND DC FIELD

The samples of milk were prepared; these were exposed to the electric field strength of 450 V/cm and 500 V/cm, in AC and DC field separately. After duration of two minutes each sample was removed, collected, transferred and spread on to an agar growth medium. The samples were left for twenty-four hours in an incubation chamber and then the microbial colony growths were counted. One sample of each acted as control, to which no electric field was applied.

2.4 THE EFFECT OF THICKNESS OF THE PLATES

The samples of milk were prepared; each of these was exposed to constant electric field strength of 950 V/cm. The thickness of the electric plate was varied for each, whilst the separation distance between the plates remained constant at 5mm. After an interval of two minutes, each sample was removed, collected, transferred and spread on to an agar growth medium. The samples were left for twenty-fours in an incubation chamber and then the microbial colony growths were counted. One sample of each acted as control, to which no electric field was applied.

2.5 ELECTROPORATION OF CELL MEMBRANE DUE TO PEF

Part I – The Cell cytoplasm is surrounded by a thin semi-permeable layer, the cell membrane. The membrane acts as a semi-permeable barrier for the intra- and extracellular transport of ions and macromolecules. This membrane is a phospholipid bilayer with a thickness of 5 nanometer (nm). By exposing the cell to a pulsed electrical field the cell membrane becomes punctured. The membrane is punctured with small pores of 50-100nm each. This effect is called electroporation, which is the loss of the cell's barrier function allowing access to the cell's valuable contents.

Electroporation takes places after 1 microsecond (μ s). The process is quick, flexible, and energy-efficient and because heat is minimized, products have a longer shelf life whilst maintaining better nutritional value than with traditional food processing techniques.

Pulsed Electric Field (PEF) or electroporation works by puncturing the cell membrane, whatever the size of cell. The process is targeted, gentle and clean. As a result, we measure dramatic increases in yield, the preservation of pigments, antioxidants and vitamins. PEF makes healthy products last longer.

A cell dimension can be 20-100 micrometer (μ m), a bacteria dimension 2-10 micrometer (μ m). Pores can have a size of 50-100 nanometer (nm) each. These small pores will be more stable when the cell membrane is subjected to more pulses. Membrane disruption occurs when the induced membrane potential exceeds a critical value of 1 volt in many cellular systems, which

corresponds to a high applied external electric field. Electroporation is irreversible above a certain threshold.

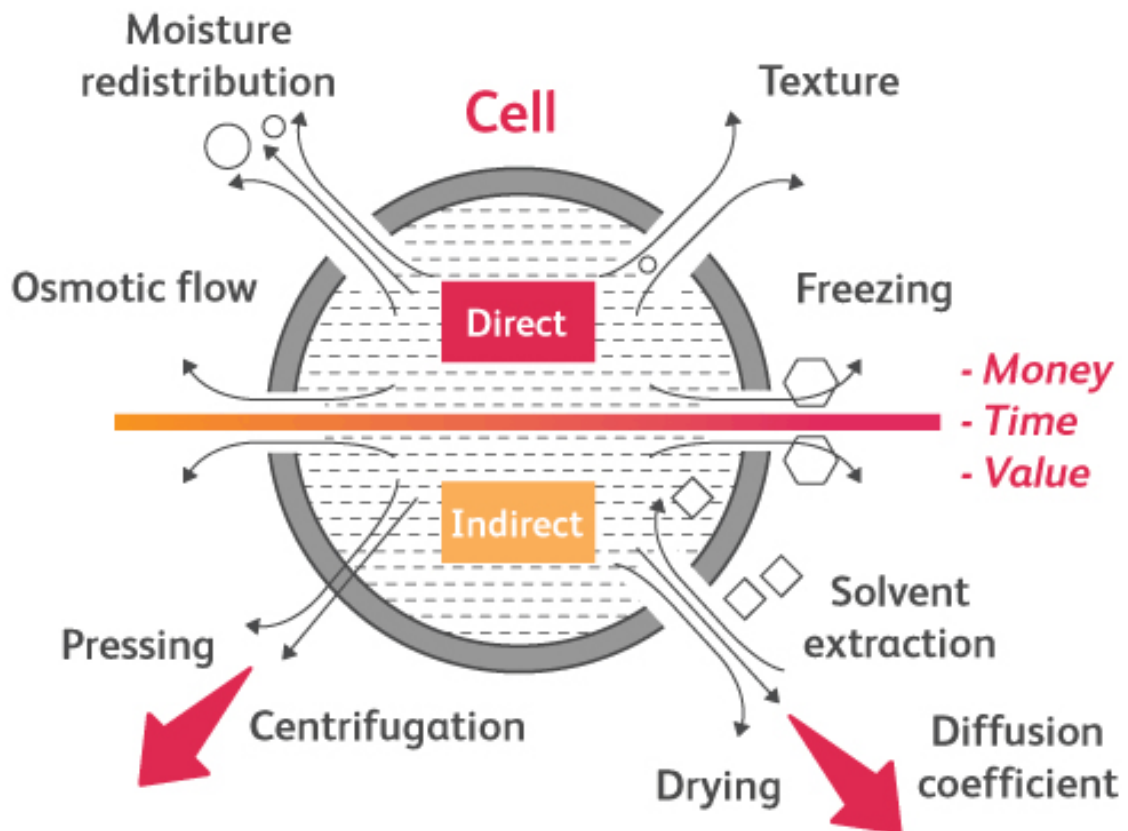


Figure 2.5.a Electroporation affecting a living cell

Part II - Kinetics of Microbial Inactivation for Alternative Food Processing Technologies - Pulsed Electric Fields

Pulsed electric field (PEF) processing involves the application of pulses of high voltage to foods placed between two electrodes. PEF treatment is conducted at ambient, sub-ambient, or slightly above ambient temperature for less than 1 s, and energy loss due to heating of foods is minimized. For food quality attributes, PEF technology is considered superior to traditional heat

treatment of foods because it avoids or greatly reduces the detrimental changes of the sensory and physical properties of foods).

Although some studies have concluded that PEF preserves the nutritional components of the food, effects of PEF on the chemical and nutritional aspects of foods must be better understood before it is used in food processing

Some important aspects in pulsed electric field technology are the generation of high electric field intensities, the design of chambers that impart uniform treatment to foods with minimum increase in temperature, and the design of electrodes that minimize the effect of electrolysis.

The large field intensities are achieved through storing a large amount of energy in a capacitor bank (a series of capacitors) from a DC power supply, which is then discharged in the form of high voltage pulses. Studies on energy requirements have concluded that PEF is an energy-efficient process compared to thermal pasteurization, particularly when a continuous system is used. [13]

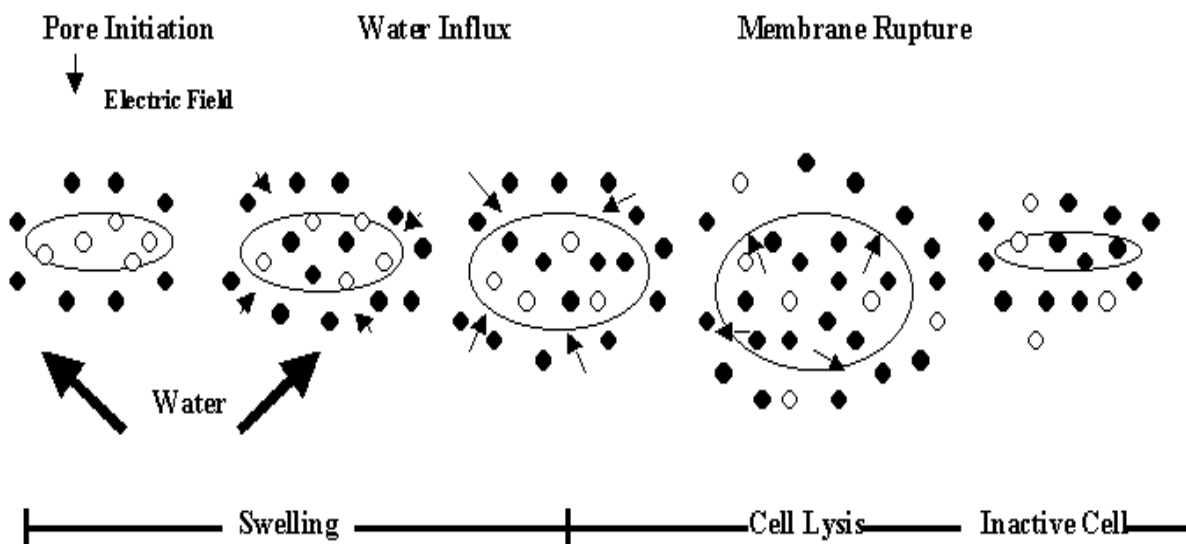


Figure 2.5.b Electroporation effecting a living cell, step by step

2.6 DERIVATION OF $E=V/d$

We know, the region or area where a charged object experiences an electric force is called as electric field. So:

$$E = q \backslash F$$

$$F = q \times E$$

Again, the amount of work required to move an electric charge from a reference point to a specific point against electric field is called as electric potential.

That is,

$$V = E \backslash q$$

Further, work is defined:

$$W = F \times d$$

If we substitute,

$$W = q \times E \times d \quad \dots \dots \dots \text{equation (1)}$$

$$W = -P \times E \quad \dots \dots \dots \text{equation (2)}$$

Equating equations (1) and (2) and dividing by q,

$$\frac{q \times E \times d}{q} = \frac{-P \times E}{q}$$

$$E \times d = -V$$

$$E = \frac{-V}{d}$$

When applied strictly to a parallel plate capacitor, however, this expression is often used in a slight different form. Traditionally, in discussions of such a capacitor, the potential difference between the plates is referred to by using the symbol V to denote the amount by which the higher potential exceeds the lower potential. $V = V_a - V_b$.

Thus,

$$E = -v \backslash d$$

Or,

$$E = -(Vb - Va) \backslash d$$

Or,

$$E = (Va - Vb) \backslash d$$

Or,

$$E = V \backslash d$$

(Proved)

NOTE: All symbols have their usual meanings.

CHAPTER 3: EXPERIMENTAL SETUPS

3.1.0 THE ELECTRICAL SYSTEM SETUP AND THE EQUIPMENT USED

- a. Variable DC supply unit 0-250V
- b. Variable AC supply unit 0-470V
- c. Multimeter (Voltmeter)~Custom made high voltage AC supply unit 0-900V
 1. AC supply source 220 V
 2. Switches
 3. Fuse
 4. Resistors
 5. Variable Registers
 6. Step up transformer 220/900V, 50Hz, 3 A
 7. Insulation
 8. Various Test Chambers

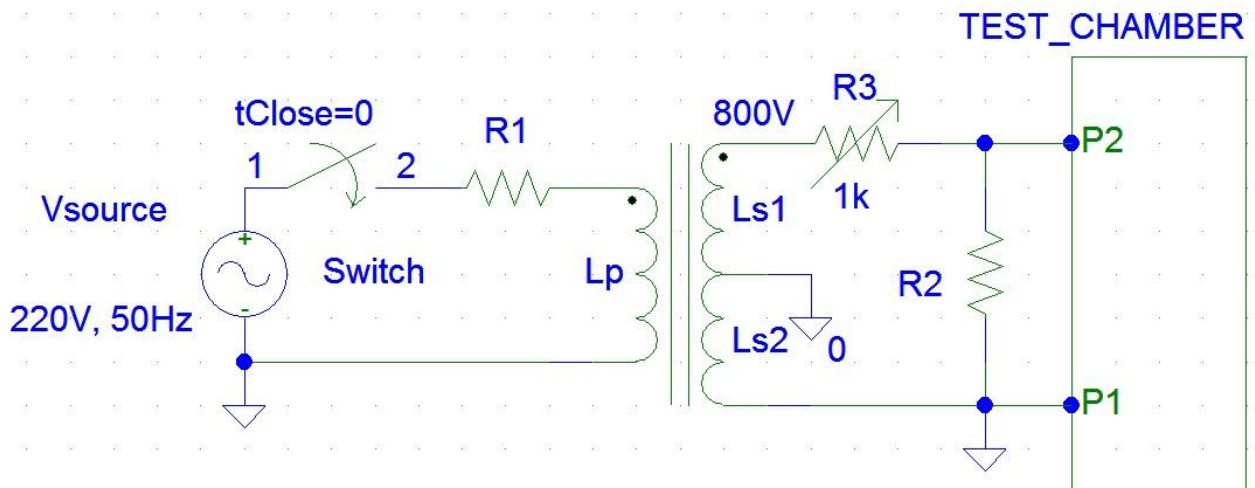


Figure 3.1.a High voltage variable AC supply unit 0-900V, 50Hz

Testing of the custom made variable AC supply at fixed 400V and at 800 V

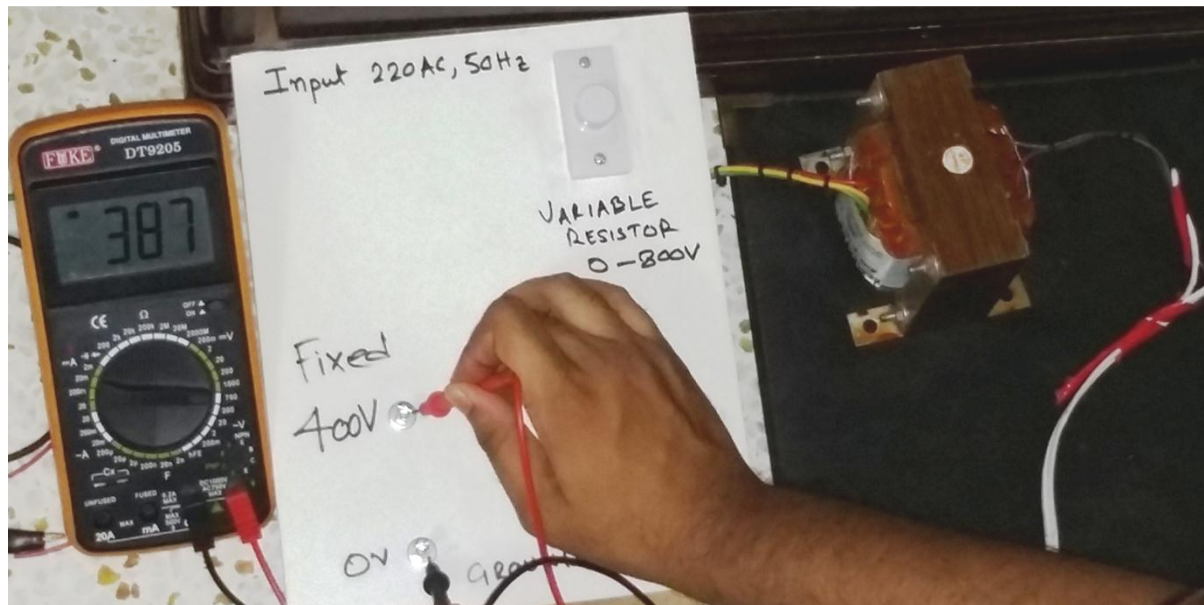


Figure 3.1.b testing of the fixed AC voltage supply unit (400V highest)

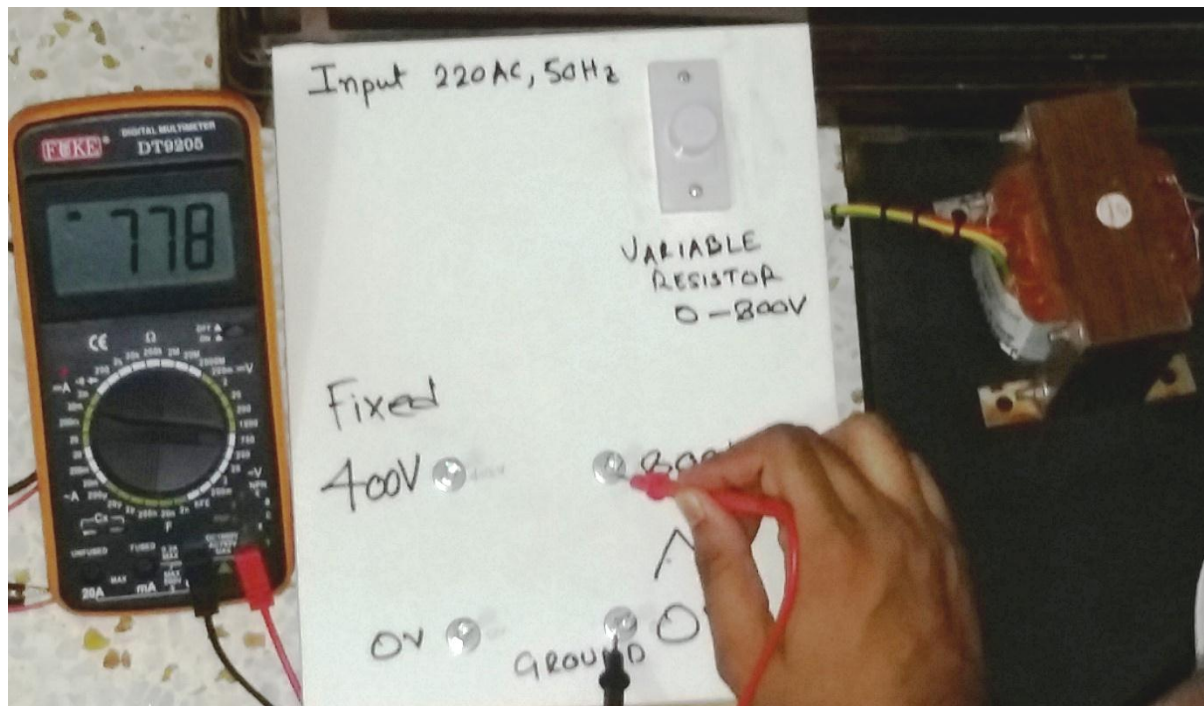


Figure 3.1.c testing of the variable AC voltage supply unit (0 ~ 800V)

3.1.1 TESTING THE RATING OF A TRANSFORMER

At first the input and output voltage of the transformer was measured by digital multimeter. The transformer was plugged in and the knob of the multimeter was placed in 1000V AC. The input voltage shown was 228V and the output voltage was 785V at two terminals. The center tapped voltage measured was 397V.

Then the transformer was plugged out to measure the resistance of the primary winding and secondary winding. The knob of the transformer was then placed at 20k Ω . The resistance at the primary winding measured was 15k Ω . Then the knob was switched to 200 Ω to measure the resistance of the secondary winding. The resistance at the secondary winding shown was 175 Ω .

With the values of voltages and resistances the input and output currents were also measured by using Ohm's law $V=IR$. The input current was found as 0.015A. The center tapped output current was attained as 2.268A and the output current at two terminals was found as 4.485A. This is how the step up transformer was measured [14].

3.2.0 THE BIOLOGICAL SYSTEMS

3.2.1 THE AGAR GEL PREPARATION

Agar: Agar is a type of gel derived from red algae which is used in biological culture media and thickener in foods. Example:

- a. m-FC agar: m-FC Agar is used with rosolic acid for the detection and enumeration of fecal coli forms by membrane filtration.
- b. Cetrimide agar: Cetrimide agar is a type of agar used for the isolation of gram-negative bacterium, *Pseudomonas aeruginosa*.

In this experiment, out of various types of agar EMB and MacConkey agar are used as the objective of the experiment is to select the gram negative bacteria and differentiate the coli forms and fecal coli forms.

3.2.2 PREPARATION OF EMB AGAR

Eosin Methylene Blue Agar is a both selective and differential culture medium. It is selective culture medium for gram-negative bacteria (selects against gram positive bacteria) and is commonly used for the isolation and differentiation of coli forms and fecal coli forms.

Procedure:

EMB agar is prepared by measuring the required amount of powder in weighing machine and then mixed with the distilled water in a conical flask. For 1000 ml distilled water the required EMB agar is 36 g. Then it is placed in a Bunsen burner and stirred with a glass rod continuously while heating for 15-20 minutes until it is boiled and dissolved. Afterwards, the conical flask is removed from the glass burner and cooled for 5 minutes before it is covered with an aluminum foil and labeled.

Then it is placed in autoclave in 121°C for 15 minutes. The conical flask is displaced from autoclave when the temperature comes down to 80°C. Later on, the agar is prepared for pouring into Petri dishes. For 1 Petri dish (100mm × 15mm) the required agar is 30 ml, for 20 Petri dishes the required agar is 600 ml. For 1 Petri dish (60mm × 12mm) the required agar is 20 ml, for 20 Petri dishes the required agar is 400 ml. To pour the EMB agar, the Petri dishes are kept in laminar air flow cabinet half opened. Subsequently, the agar is poured in the Petri dishes from the conical flask and kept for 10 minutes for solidification. Finally, all the Petri dishes are taken and refrigerated before putting into incubator [15].

3.2.3 PREPARATION OF MACCONKEY AGAR

MacConkey Agar is a selective and differential culture media commonly used for the isolation of enteric Gram-negative bacteria. MacConkey agar is used for the differentiation of lactose fermenting gram-negative bacteria and to differentiate bacteria that ferment sugars other than lactose.

Procedure:

MacConkey agar is prepared by measuring the required amount of powder in weighing machine and then mixed with the distilled water in a conical flask. For 1000 ml distilled water, required MacConkey agar is 50 g. Then it is placed in a Bunsen burner and stirred with a glass rod continuously while heating for 15-20 minutes until it is boiled and dissolved. Afterwards, the conical flask is removed from the glass burner and cooled for 5 minutes before it is covered with an aluminum foil and labeled.

Then it is placed in autoclave in 121°C for 15 minutes. The conical flask is displaced from autoclave when the temperature comes down to 80°C. Later on, the agar is prepared for pouring into Petri dishes. For 1 Petri dish (Big size) the required agar is 30 ml, for 20 Petri dishes the required agar is 600 ml. For 1 Petri dish (Medium size) the required agar is 20 ml, for 20 Petri dishes the required agar is 400 ml. To pour the EMB agar, the Petri dishes are kept in laminar air flow cabinet half opened. Subsequently, the agar is poured in the Petri dishes from the conical

flask and kept for 10 minutes for solidification. Finally, all the Petri dishes are taken and refrigerated before putting into incubator. (Murray, Baron, Jorgensen, Landry and P faller, 2007)

3.2.4 THE DIFFERENCE BETWEEN EMB AND MACCONKEY AGAR

MacConkey agar plate and EMB are both selective for gram negative enteric and coli forms. They have inhibitors to stop growth of gram positive bacteria such as *staphylococcus*. Both media are also differential for fermenters. MacConkey agar plate contains lactose but EMB also contains sucrose in addition to lactose. Moreover, MacConkey agar plate has bile salt that inhibits gram positive bacteria, but EMB contains aniline dyes (methylene blue and eosin) [16].

3.2.5 SAFETY PROTOCOL

While working in a microbiology laboratory, protocols and safety measures must be followed.

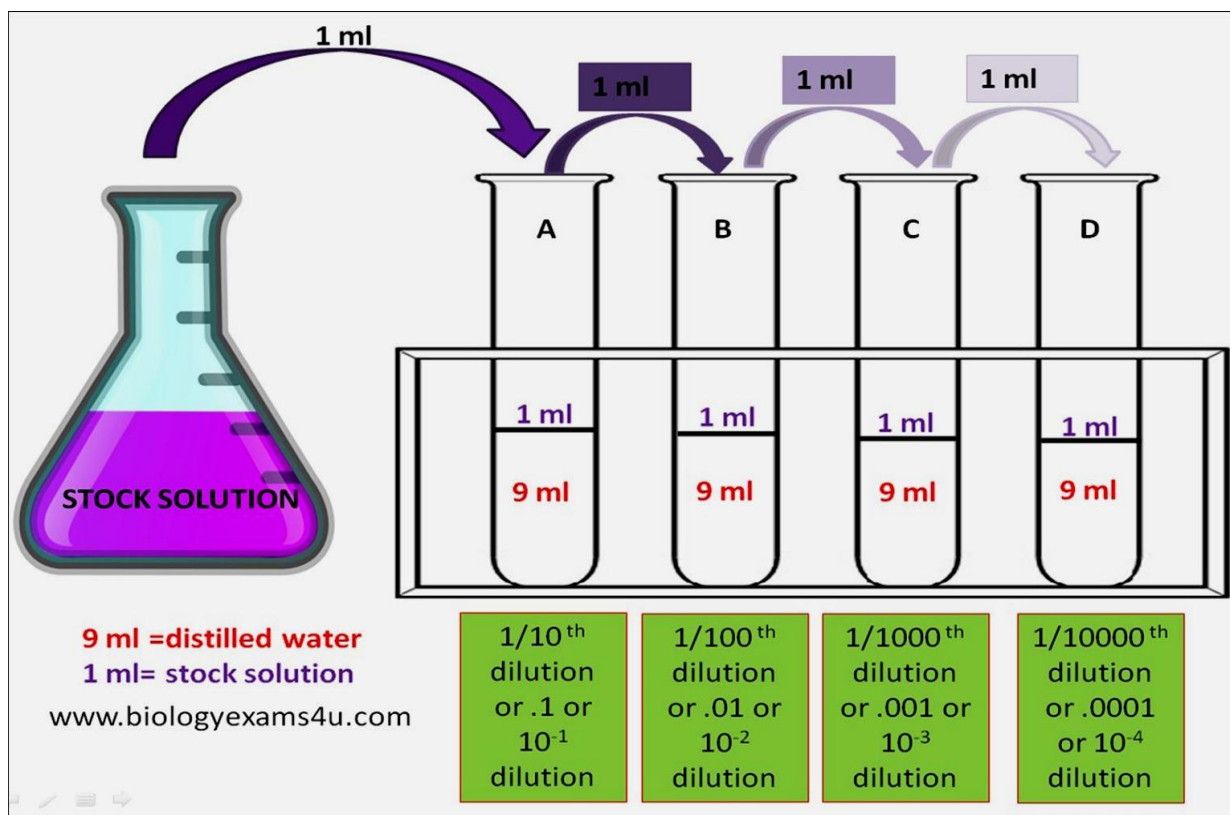
- Wearing apron while performing the experiment
- Sterilizing the equipments and materials
- Disinfecting work area before and after use
- Washing the hands properly
- Proper labeling should be done

3.2.6 PREPARATION OF SALINE SOLUTION

At first required amount of sodium chloride (NaCl) is measured using a weighing machine and introduced into a beaker with the distilled water. For 100 ml distilled water 0.9 gm NaCl is used, for 1000 ml distilled water 9 g NaCl is used. Later on, the powder is dissolved with the help of a stirrer. Finally, the solution is transferred into required test tubes using pipette of 9 ml and labeled. Now, the saline solution is prepared. For 1 test tube required saline solution is 9 ml, for 10 test tubes required saline solution is 90 ml [17].

3.2.7 PREPARATION OF SAMPLE USING SERIAL DILUTION METHOD

Each sample is prepared using the serial dilution method which is a stepwise dilution of a substance in a solution where 1 ml of sample from stock solution which is taken using pipette in one test tube having distilled water of 9 ml and then transferring it into another test tube and this series is carried on till the required concentration is achieved.



Applications: This method is used in experimental sciences including Biochemistry, Microbiology, Pharmacology and Homeopathy. [18].

3.2.8 PROCEDURE FOR COLONY COUNT IN PETRI DISHES

Colonies are counted with naked eye using marker in Petri dishes. Each colony is marked by putting dot onto the glass cover using marker. Thus, the total number of growth is counted from the dots marked on the Petri dishes. For varying voltage and varying time the growth of bacteria are different. Finally, all the data are recorded for further analysis. The Colony forming units (CFU) is used to calculate the number of bacterial cells, yeasts and other microorganisms in a sample [19].

Formula for colony forming units (CFU):

$$\text{CFU/ml} = \frac{\text{Number of colonies}}{\text{ml plated} \times \text{Total dilution factor}}$$

Example:

If the number of colonies is 110, the used sample for spreading is 0.1 ml per plate and the dilution factor is 10^{-4} ,

$$\begin{aligned}\text{CFU/ml} &= 110 / (0.1\text{ml} \times 10^{-4}) \\ &= 1100 \times 10^4 \\ &= 1.1 \times 10^7 \text{ microorganism count}\end{aligned}$$

CHAPTER 4: EXPERIMENTS AT DIFFERENT TESTING CONDITIONS

4.1.1 EXPERIMENT 1: SAMPLES AT DIFFERENT AC FIELD LENGTH

4.1.2 EQUIPMENTS USED

- a. Variable AC generator(0 – 800V, 50Hz)
- b. Digital multimeter (voltmeter)
- c. Stop watch
- d. Specially designed test chamber - parallel plates (5mm separation).
- e. Glass slides
- f. Pipettes(1000ul and 200ul)
- g. Test tubes
- h. EMB agar gel
- i. 0.9% saline solution
- j. Petri dishes
- k. Incubator
- l. Autoclave
- m. Refrigerator

4.1.3 EXPERIMENTAL PROCEDURE

The EMB agar gel and the saline solution were prepared the day before the experiment and sterilized in an autoclave for 15 minutes. The test tube containing the saline solution was then stored at room temperature. The agar was then spread on the Petri dishes and left inside the refrigerator. On the day of the experiment the dishes are removed from the refrigerator and left in the incubator for 2 hours. Milk samples, fruit juice sample, tap water sample and sugarcane extract were prepared in the saline solution of 0.9% dilution using the serial dilution method. The final samples had a dilution factor of 10^{-4} .

The special test chamber was then sterilized and made ready. The variable AC source voltage was tested and noted using a digital multimeter and then connected to the test chamber. From the test tube containing the milk sample (10^{-4}), 200ul of the sample was then collected using a micro pipette and transferred to a clean glass slide. The glass slide was then placed inside the test chamber, the power supply was turned on and the voltage of 350V was applied for 2 minutes.

The EMB agar Petri dish was removed from the incubator and then 100ul of the test sample was collected using pipette and spread on it. The Petri dish was then again placed inside the incubator for 24 hours and then the microbial colony was counted. A sample which was left unexposed acted as the control. The experiment was repeated for 400V, 450V, 470V and 800V, with the samples of fruit juice, tap water and sugarcane extract.



Figure 4.1.a Testing and placing of the glass slide position between two plates



Figure 4.1.b removing the glass slide, placing the sample. Notice the separation distance

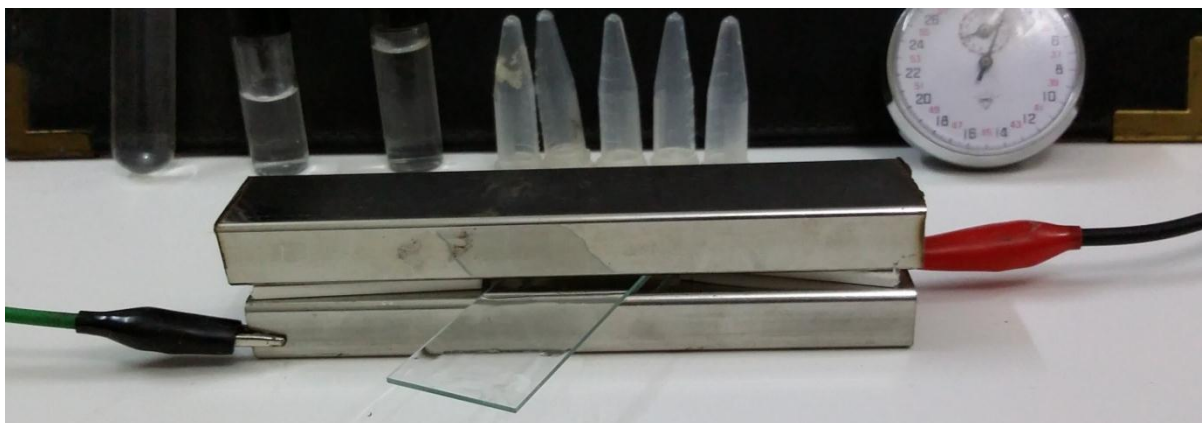


Figure 4.1.c glass slide with sample, placed into position and the power is switched on

4.2.1 EXPERIMENT 2: EFFECT OF NUMBER OF PULSES

4.2.2 Equipments used

- a. Variable AC generator
- b. Digital multimeter (voltmeter)
- c. Stop watch
- d. Specially designed test chamber – parallel plate holder (5mm separation).
- e. Glass slides
- f. Pipettes(1000ul and 200ul)
- g. Test tubes
- h. Durham tubes(5mm)
- i. EMB agar gel
- j. 0.9% saline solution
- k. Petri dishes
- l. Incubator
- m. Autoclave
- n. Refrigerator

4.2.3 EXPERIMENTAL PROCEDURE

The EMB agar gel is prepared the day before the experiment, it spread on the Petri dishes and left inside the refrigerator. On the day of the experiment, the dishes were removed from the refrigerator and left in the incubator for 2 hours. The milk sample was prepared using 0.9% saline solutions using the serial dilution method. The final dilution factor was 10^{-4} .

The test chamber having two parallel plates was then sterilized and made ready. The variable AC source voltage was tested and noted using a digital multimeter and then connected to the test chamber. From the test tube containing the milk sample (10^{-4}), 200ul of the sample was then collected using a pipette and then transferred into Durham tubes. In total, five Durham tubes each containing 200ul of the sample was placed between the parallel plates. Since the supply

voltage was 4000V/ 50Hz, therefore the number of AC pulses was 3,000 pulses per minute. After time intervals of 1,2,3,5 and 10 minutes each tube was removed, 100ul of its content extracted by pipette and spread on the EMB agar Petri dish which was removed from the incubator earlier.

The Petri dish was then again placed inside the incubator for 24 hours and then the microbial colony was counted on the next day. A sample was left unexposed which acted as the control. The experiment was repeated for 800V.



Figure 4.2.a Equipments: plate holder, Durham tubes, vials, pipette tips, stopwatch

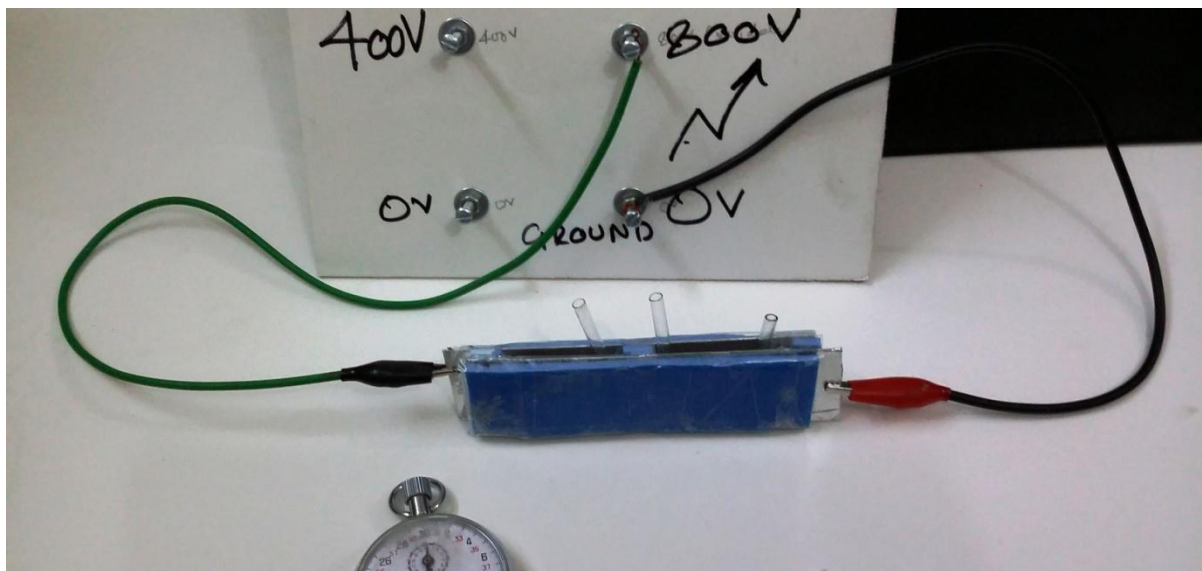


Figure 4.2.b the set up is connected to the supply; Durham tubes filled with samples are Placed in the parallel plate holder

4.3.1 EXPERIMENT 3: COMPARING AC WITH DC FIELD

4.3.2 EQUIPMENTS USED

- a. Variable AC generator (0 – 500V, 50Hz)
- b. Variable DC generator (0 – 500V)
- c. Digital multimeter (voltmeter)
- d. Stop watch
- e. Parallel plate test chamber
- f. Glass slides
- g. Pipettes(1000ul and 200ul)
- h. Test tubes
- i. EMB agar gel
- j. Petri dishes
- k. 0.9% saline solution
- l. Incubator
- m. Autoclave
- n. Refrigerator

4.3.3 EXPERIMENTAL PROCEDURE

The EMB agar gel is prepared the day before the experiment, it spread on the Petri dishes and was left inside the refrigerator. On the day of the experiment, the dishes were removed from the refrigerator and left in the incubator for 2 hours. The milk sample was prepared using 0.9% saline solutions using the serial dilution method. The final dilution factor was 10^{-4} .

The test chambers used in this experiment had two parallel plates with separation of 5mm. These were then sterilized and made ready. The variable AC source voltage and the variable DC source voltage were tested and noted using a digital multimeter and then connected to the test chambers respectively. A voltage of 225V was applied. From the test tube containing the milk sample (10^{-4}), 200ul of the sample was then collected using a micro pipette and transferred to clean

glass slides. One was exposed to the DC field whilst the other was exposed to the AC field for 3 minutes, simultaneously.

The EMB agar Petri dish was removed from the incubator and then 100ul of the test sample from each were collected using pipette and spread on the dishes. These were then again placed inside the incubator for 24 hours and then the microbial colony was counted. A sample which was left unexposed acted as the control. The experiment was repeated for 250V.



Figure 4.3.a the setup is very similar to experiment 4.1.1, however once it is connected to the variable DC supply and the other time to a variable AC supply

4.4.1 EXPERIMENT 4: EFFECT OF THICKNESS OF THE PLATES

4.4.2 EQUIPMENTS USED

- a. Variable AC generator (0 – 800V, 50Hz)
- b. Digital multimeter (voltmeter)
- c. Stop watch
- d. Parallel plate test chamber
- e. Thin glass slides
- f. Pipettes(1000ul,200ul)
- g. Test tubes
- h. EMB agar gel
- i. Petri dishes
- j. 0.9% saline solution
- k. Incubator
- l. Autoclave
- m. Refrigerator

4.4.3 EXPERIMENTAL PROCEDURE

The EMB agar gel is prepared the day before the experiment, it spread on the Petri dishes and left inside the refrigerator. On the day of the experiment, the dishes were removed from the refrigerator and left in the incubator for 2 hours. The milk sample was prepared using 0.9% saline solutions using the serial dilution method. The final dilution factor was 10^{-4} .

In this experiment the plates of varying thickness of 1mm to 16mm were used and the average separation between the plates was 5mm. The voltage applied was constant at 475V. For the first case, plates of 1mm thickness were used. From the test tube containing the milk sample (10^{-4}), a volume of 200ul of the sample was then collected using a micro pipette and transferred to clean glass slides and placed between the plates for 3 minutes.

The EMB agar Petri dish was removed from the incubator and then 100ul of the test sample from each were collected using pipette and spread on the dishes. These were then again placed inside

the incubator for 24 hours and then the microbial colony was counted. A sample which was left unexposed acted as the control. The experiment was then repeated for plate thicknesses: 2, 6, 10, 12 and 16 mm.

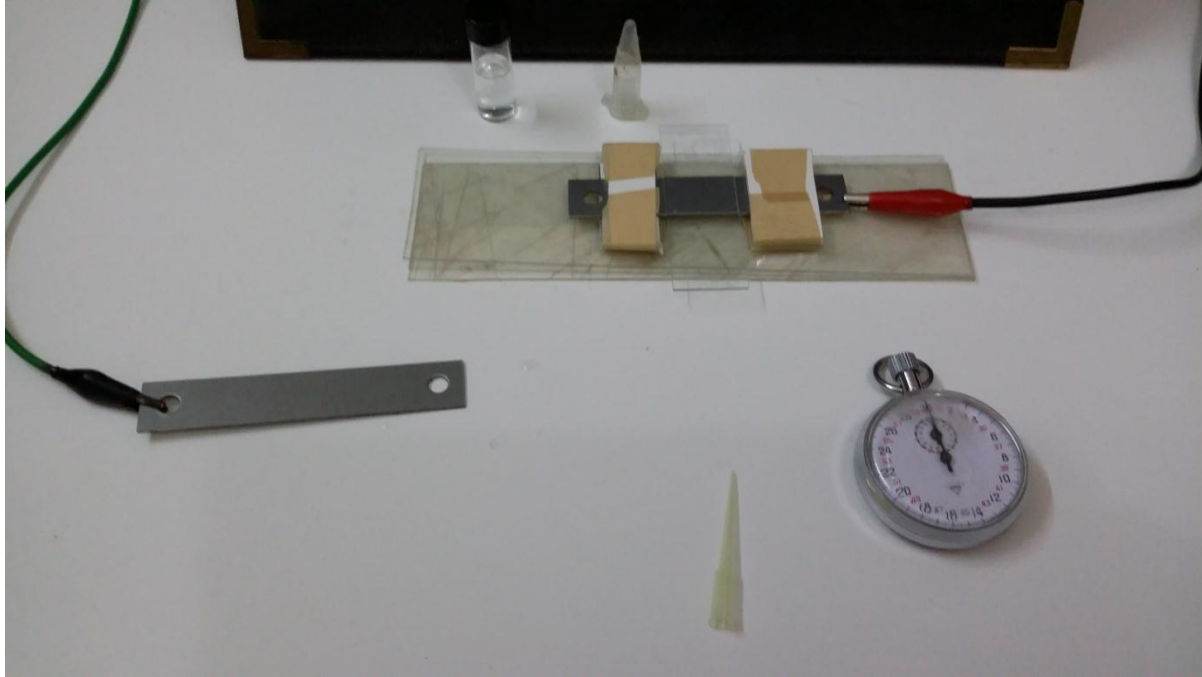


Figure 4.4.a the equipments and the general setup

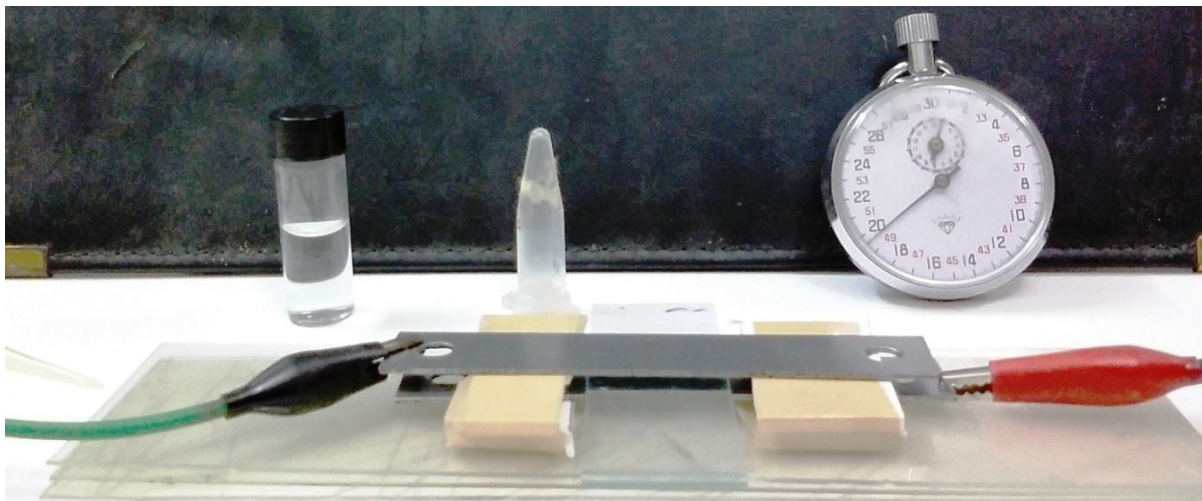


Figure 4.4.b the experiment with one single plate (1mm thickness)



Figure 4.4.c increasing thickness of the plates to 16mm



Figure 4.4.d placing the sample on the glass slide and connecting the 16mm plates to power supply

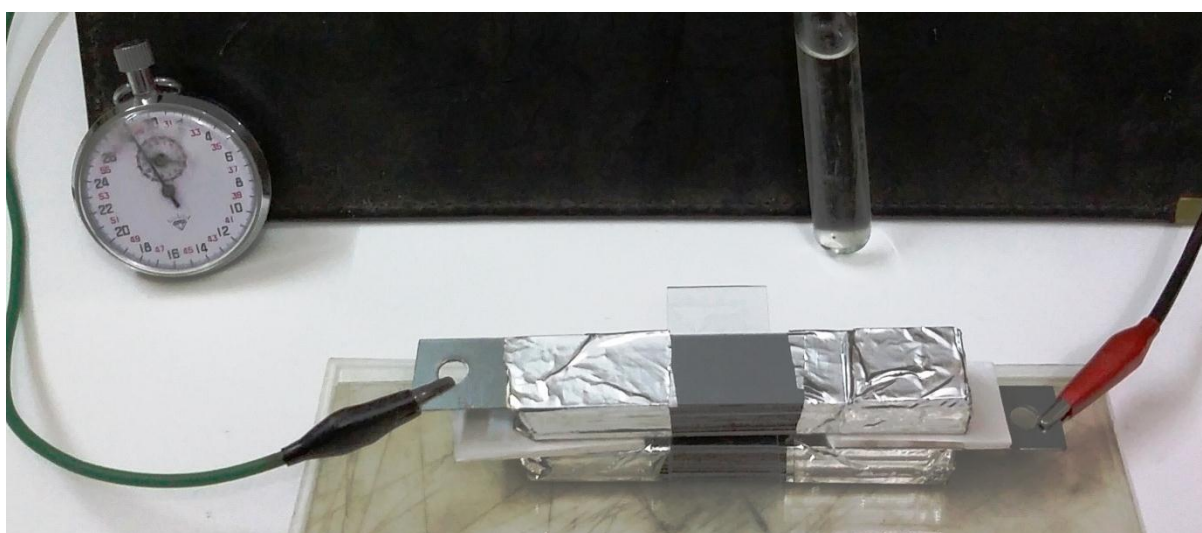


Figure 4.4.e the top view of the Figure 4.4.c

4.5.1 SAFETY PROTOCOLS

4.5.2 WHILE PERFORMING THE EXPERIMENTS WITH THE ELECTRICAL EQUIPMENTS IN THE POWER LABORATORY

- A. Wearing thick soled shoes
- B. Wearing Rubber gloves
- C. Wearing safety glasses if needed
- D. While using high voltage supply, extreme care is needed
- E. Keeping equipments dry
- F. Testing equipments before starting the experiments

4.5.3 WHILE PERFORMING THE EXPERIMENTS IN THE BIOLOGY LABORATORY

- Wearing laboratory coat while performing the experiment.
- Sterilizing the equipments and materials.
- Disinfecting work area before and after use.
- Washing the hands properly.
- Proper labeling should be done.

CHAPTER 5: DATA AND RESULTS

The data from the experiments conducted are tabulated below and their respective graphs are plotted showing the microbial count. The experiential conditions are also stated.

5.1.0 EXPERIMENTAL CONDITIONS

Plate separation	0.5 cm	pH	7
Sample (tip) volume	0.1 ml	Temperature	24 °C
Dilution factor	10^{-4}	Supply	220, 50Hz
Saline solution	0.9%	Pressure	1 atm

5.1.1 SAMPLE DATA AT DIFFERENT AC FIELD LENGTH SHOWING THE COLONY COUNT

<i>[Table]: 5.1.1 Samples at different AC field strength</i>					
Sample:	Raw milk_1	Raw milk_2	Fresh Juice	Tap water	Sugarcane
Applied Voltage (V)	Colony Count	Colony Count	Colony Count	Colony Count	Colony Count
0	108	203	370	478	483
350	94	181	282	452	469
400	81	163	263	437	420
450	67	139	258	390	350
470	54	110	240	315	289
600	33	71	227	264	218
800	21	46	201	227	176

5.1.2 SAMPLE DATA AT DIFFERENT AC FIELD LENGTH SHOWING THE MICROBIAL COUNT

<i>[Table] 5.1.2 Samples at different AC field strength</i>					
Sample:	Raw milk_1	Raw milk_2	Fresh Juice	Tap water	Sugarcane
Electric field (V/cm)	Microbial count	Microbial count	Microbial count	Microbial count	Microbial count
0	1.08E+07	2.03E+07	3.70E+07	4.78E+07	4.83E+07
700	9.40E+06	1.81E+07	2.82E+07	4.52E+07	4.69E+07
800	8.10E+06	1.63E+07	2.63E+07	4.37E+07	4.20E+07
900	6.70E+06	1.39E+07	2.58E+07	3.90E+07	3.50E+07
940	5.40E+06	1.10E+07	2.40E+07	3.15E+07	2.89E+07
1200	3.30E+06	7.10E+06	2.27E+07	2.64E+07	2.18E+07
1600	2.10E+06	4.60E+06	2.01E+07	2.27E+07	1.76E+07

5.2.0 EXPERIMENTAL CONDITIONS

Plate separation	0.5 cm	pH	7
Sample (tip) volume	0.1 ml	Temperature	23 °C
Dilution factor	10 ⁻⁴	Supply	220V, 50Hz
Saline solution	0.9%	Pressure	1 atm

5.2.1 SAMPLE DATA SHOWING THE EFFECT OF NUMBER OF PULSES ON THE COLONY AND THE MICROBIAL COUNT

<i>Table [5.2.1] Effect of number of pulses</i>							
Electric field (V/cm)	Time (min)	No. of pulses	Colony Count	Microbial count	Electric field (V/cm)	Colony Count	Microbial count
0	0	0	344	3.44E+07	0	127	1.27E+07
800	1	3000	310	3.10E+07	1600	113	1.13E+07
800	2	6000	289	2.89E+07	1600	98	9.80E+06
800	3	9000	263	2.63E+07	1600	86	8.60E+06
800	5	15000	235	2.35E+07	1600	72	7.20E+06
800	10	30000	211	2.11E+07	1600	43	4.30E+06

5.3.0 EXPERIMENTAL CONDITIONS

Plate separation	0.5 cm	pH	7
Sample (tip) volume	0.1 ml	Temperature	25 °C
Dilution factor	10^{-4}	Supply 1 AC	0 - 250V, 50Hz
Saline solution	0.9%	Supply 2 DC	0 - 250V

5.3.1 SAMPLE DATA SHOWING THE EFFECTS OF AC FIELD AND DC FIELD ON THE COLONY AND THE MICROBIAL COUNT

<i>Table [5.3.1] AC field versus DC field</i>				
Electric field (V/cm)	AC colony count	AC microbial count	DC colony count	DC microbial count
0	78	7.80E+06	78	7.80E+06
450	60	6.00E+06	64	6.40E+06
500	58	5.80E+06	54	5.40E+06

5.4.0 EXPERIMENTAL CONDITIONS

Plate separation	0.5 cm	pH	7
Sample (tip) volume	0.1 ml	Temperature	22 °C
Dilution factor	10^{-5}	Supply	220V, 50Hz
Saline solution	0.9%	Pressure	1 atm

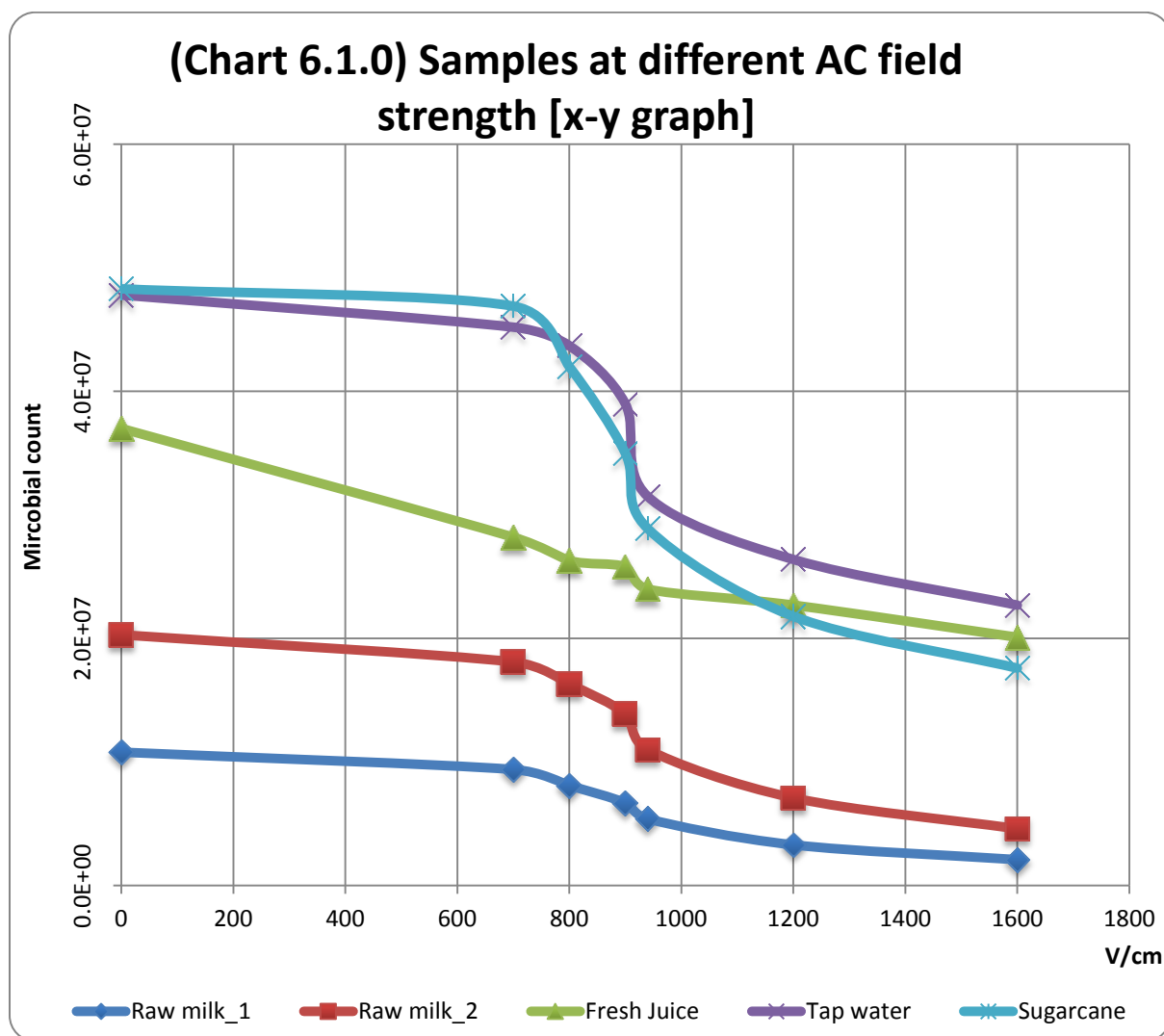
5.4.1 SAMPLE DATA SHOWING THE EFFECT OF THE THICKNESS OF THE PLATES TO PLATE SEPARATION DISTANCE TO THE COLONY AND THE MICROBIAL COUNT

<i>Table [5.4.1] Thickness of the plates</i>					
Electric field (V/cm)	Distance between plates (mm)	Thickness of plates (mm)	Ratio of separation	Colony count	Microbial count
0	5	1	0.2	284	2.84E+08
950	5	1	0.2	268	2.68E+08
950	5	2	0.4	189	1.89E+08
950	5	6	1.2	134	1.34E+08
950	5	10	2.0	93	9.30E+07
950	5	12	2.4	75	7.50E+07
950	5	14	2.8	45	4.50E+07
950	5	16	3.2	32	3.20E+07

CHAPTER 6: ANALYSIS AND DISCUSSION

6.1.0 The effect on the microbial growth for samples exposed under variable AC fields

During the experiment six samples of each food products: raw milk, fresh mango juice, tap water and sugarcane extract were. Each of these was than exposed to the electrical field strength of: 350 V/cm, 400 V/cm, 450 V/cm, 470 V/cm, 600 V/cm and 800 V/cm respectively. The data from the Table 5.1.2 was plotted on the graph below, Chart 6.1.1.



From the Chart 6.1.0 it can be said that the microbial count decreases with the increasing electric field strength. Raw milk samples (1 and 2) show a very similar pattern to each other, the fresh

mango juice also follow the same pattern. Tap water and the sugarcane extract, though initially having high microbial counts, show a rapid decrease in the count at 900 V/cm. The general result or the relationship may be summarized as:

[Equation 6.1.0]

$$\text{Microbial count (N)} \propto \frac{1}{\text{Applied electric field (E)}}$$

It is interesting to note that, for each sample the final microbial count or survival rate was different. Therefore, in order to do further analysis, from this point onwards final microbial count rate would be either expressed as the percentage of Survival rate (SR) of the microorganisms or the as percentage of microbes neutralized by the electric field i.e. Lethality rate (LR). The relationship between Survival rate (SR) and Lethality rate (LR) is stated below:

[Equation 6.1.1]

$$\text{Lethality rate \%} = \frac{\text{Intial (control)count} - \text{Final count}}{\text{Intial (control)count}} \times 100\%$$

[Equation 6.1.2]

$$\text{Survival rate \%} = 100\% - \text{Lethality rate \%}$$

To illustrate this relationship, a condensed Table 5.1.2 is given below, showing the relationship between Lethality rate (LR) and Survival rate (SR).

<i>Table [6.1.1] Samples at different AC field strength</i>					
Sample:	Raw milk_1	Raw milk_2	Fresh Juice	Tap water	Sugarcane
Electric field (V/cm)	Microbial count	Microbial count	Microbial count	Microbial count	Microbial count
0 (control)	1.08E+07	2.03E+07	3.70E+07	4.78E+07	4.83E+07
1600	2.10E+06	4.60E+06	2.01E+07	2.27E+07	1.76E+07
Lethality rate (%)	80.6%	77.3%	45.7%	52.5%	63.6%
Survival rate (%)	19.4%	22.7%	54.3%	47.5%	36.4%
Survival ratio (S)	0.19	0.23	0.54	0.47	0.36

Thus modifying [Equation 6.1.0] and [Equation 6.1.1], we get:

[Equation 6.1.3]

$$\text{Lethality rate (L)} \propto \text{Applied electric field (E)}$$

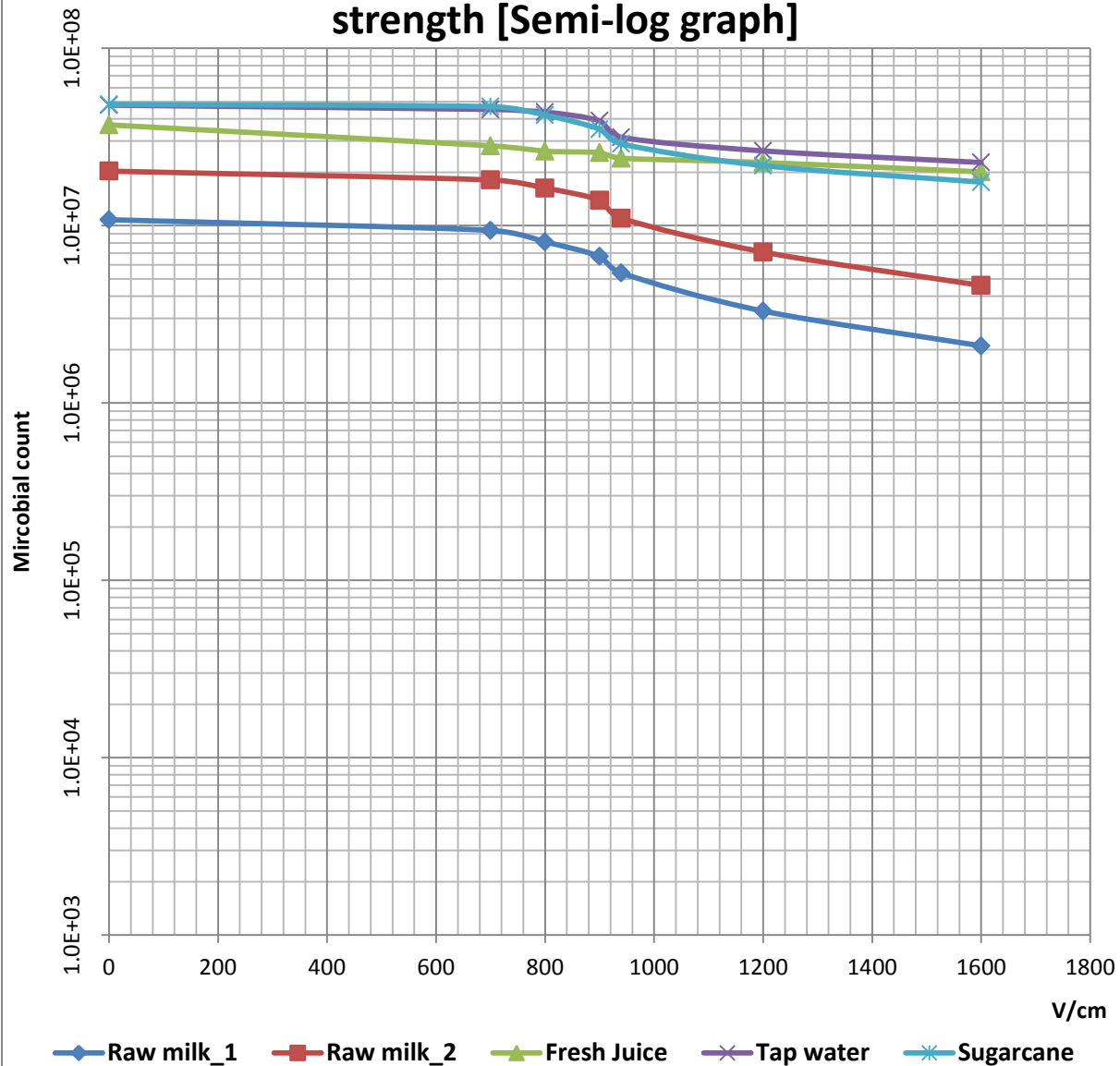
Introducing proportionality constant (K), the equation becomes:

[Equation 6.1.4]

$$\text{Lethality rate (L)} = K \cdot \text{Applied electric field (E)}$$

From Chart 6.1.0, it is challenging to do additional analysis on the data therefore a new graph, Chart 6.1.2 is drawn using a semi-log plot. The y-axis is adjusted to show the microbial count in log scale and the data lines show almost linear relationship.

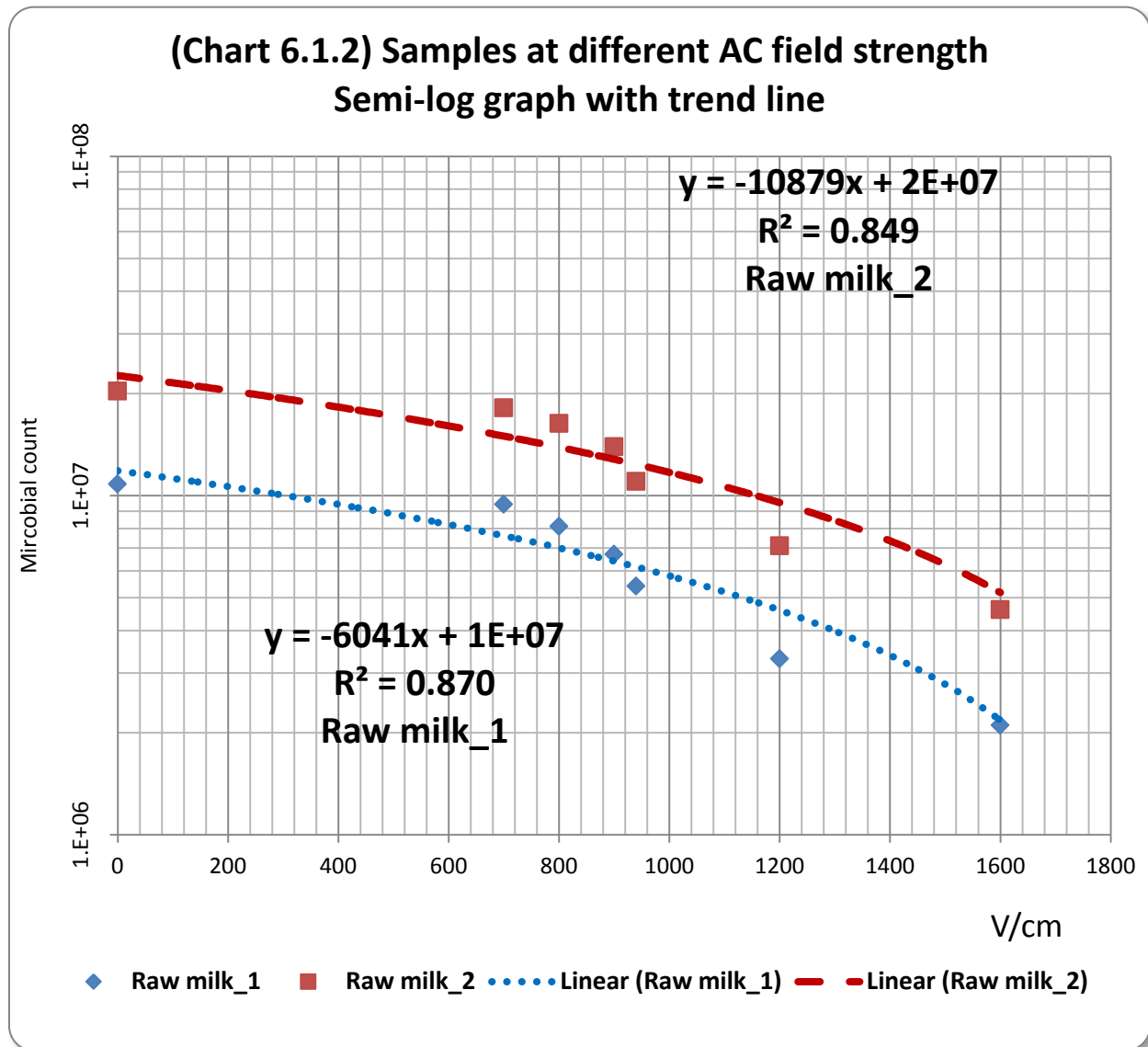
(Chart 6.1.1) Samples at different AC field strength [Semi-log graph]



For the next part, the data for the samples: raw milk_1 and raw milk_2 are chosen because of two important reasons first both are the same food product (cow milk) and secondly because the microorganisms present in them would be similar, hence these would be affected in a similar manner under the influence of an electric field. The other samples namely: tap water, mango juice and sugarcane extract would have different microorganisms present, consequently these would behave differently than the milk samples.

6.1.1 RELATIONSHIP BETWEEN THE MICROORGANISMS SURVIVAL RATIO AS A FUNCTION OF THE EFFECTIVE APPLIED ELECTRIC FIELD

The data points for samples of raw milk_1 and raw milk_2 are taken and then a best fit trend (straight) line is drawn. From that trend line analysis, the relationship between the survival rates (SR) of the microorganism as a function of the applied electric field (E) can be deduced.



Assuming straight line equation for the trend lines:

$Y = m x + C$				
The equation would be modified for the new following standard form				
Y	represents	Final microbial count	new symbol	N_f
m	represents	Regression coefficient (cm kV⁻¹)	new symbol	b_e
x	represents	Effective electric field (V/d)	new symbol	E
C	represents	Initial microbial count	new symbol	N_i

Taking natural logarithm:

$$\ln Y = - m x + \ln C$$

Rearranging and substituting:

$$\ln Y - \ln C = - m x$$

$$\ln(Y/C) = - m x$$

$$\ln\left(\frac{N_f}{N_i}\right) = - b_e E$$

N_f/N_i can also be written as the Survival ratio (S), hence finally the above equation condenses down to:

[Equation 6.1.5]

$$\ln(S) = - b_e E$$

The above equation is similar to the one proposed by Hülshager, which gives the relationship between survival ratio ($S = N / N_0$) of microorganisms and electric field:

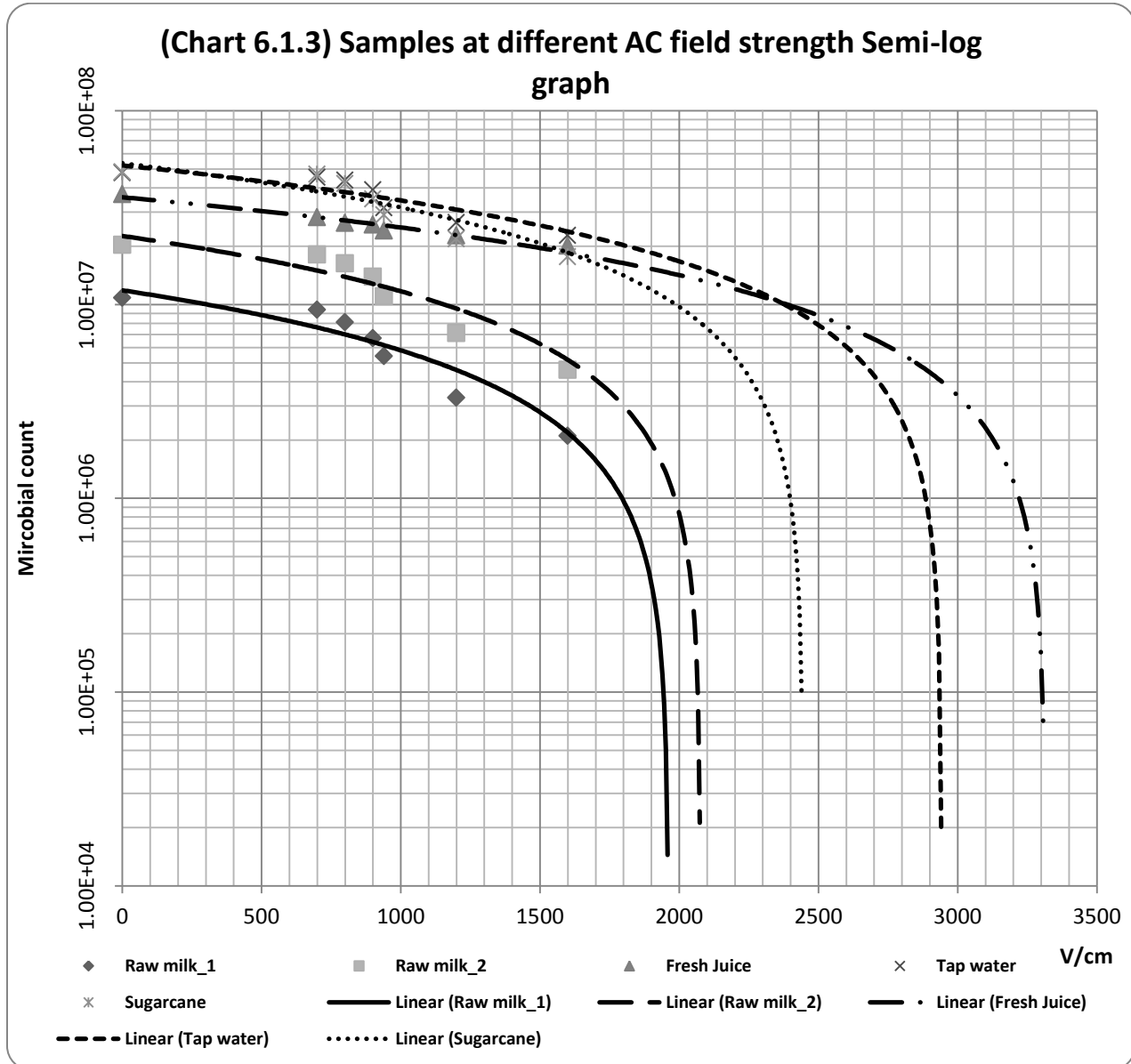
$$\ln(S) = -b_E (E - E_c).$$

Where E_c is the critical electric field obtained by extrapolating the value of E for a survival ratio of one unit. The value of E_c was found to be a function of the cell size; the larger cell, the lower the critical electric field required also established that E_c values for Gram-negative bacteria were

lower than those for Gram-positive bacteria, which would explain the lesser resistance to PEF of Gram-negative bacteria. This would also account for the Survival ratios of the samples of milk and sugarcane extract, both of which have a higher count of Gram-negative bacteria (E. Coli) than either the tap water or the fresh mango juice [7].

6.1.2 EXTRAPOLATIONS OF THE TREND LINES AND INTERPRETATIONS

Referring to the Chart 6.1.2, the trend lines of other samples can be also extrapolated further to give even a lower final microbial count. Considering the final average microbial count to be $1.0\text{E}+05$, it is clear that different samples require different amount of effective electric field.



Considering the average final Microbial count as 1.0E+05					
Sample	Raw milk_1	Raw milk_2	Fresh Juice	Tap water	Sugarcane
Initial Microbial count	1.08E+07	2.03E+07	3.70E+07	4.78E+07	4.83E+07
Electric field (V/cm) at final count	1950	2100	3300	2900	2450
Survival ratio	0.009	0.005	0.003	0.002	0.002
Survival rate %	0.9%	0.5%	0.3%	0.2%	0.2%

6.2.0 EFFECT OF NUMBER OF PULSES

A simplified Table 5.2.1 is shown below with the Survival ratio

<i>Table [6.2.1] Effect of number of pulses</i>					
Electric field (V/cm)	Time (minutes)	No. of pulses	Microbial count	Electric field (V/cm)	Microbial count
0	0	0	3.44E+07	0	1.27E+07
800	10	30000	2.11E+07	1600	4.30E+06
Survival ratio (S)			0.61	-	0.34

The Survival ratio indicates that the application of higher electric field increases the Lethality rate for the sample type of sample (raw milk). From the table it can be concluded that doubling the electric field strength will reduce the Survival ratio by about half, for the same number of pulses. This may be expressed as:

[Equation 6.2.0]

$$\text{Survival ratio (S)} \propto \frac{1}{\text{Applied electric field (E)}}$$

Introducing a proportionality constant (p, for the number of pulses) in the above equation, we get:

[Equation 6.2.1]

$$\text{Survival ratio (S)} \propto \frac{1}{\text{Number of pulses (p)}}$$

Combining the both equations

[Equation 6.2.2]

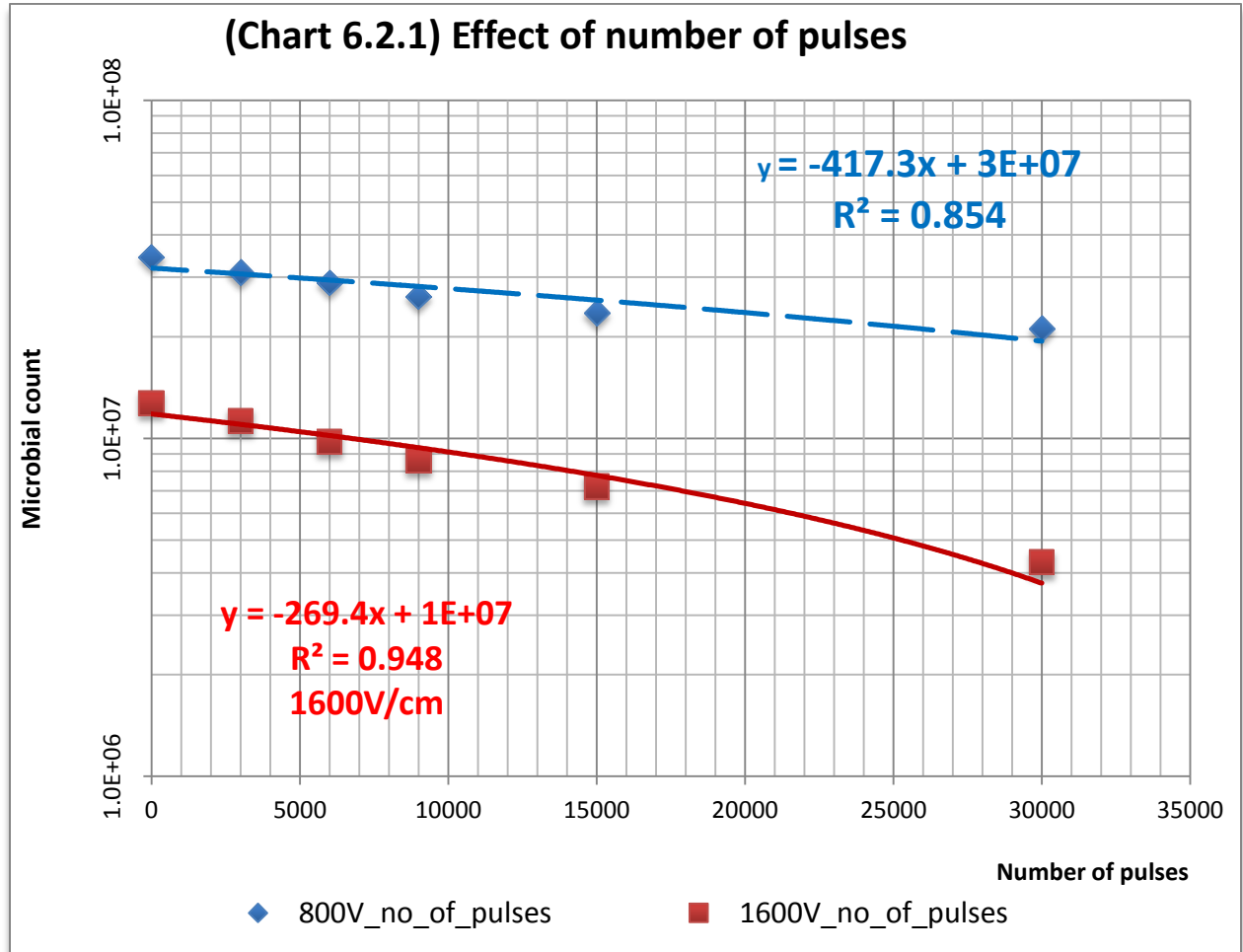
$$\text{Survival ratio (S)} \propto \frac{1}{\text{Number of pulses (p)} \times \text{Applied electric field (E)}}$$

Introducing constants we get;

$$\text{Survival ratio (S)} = \frac{k}{\text{Number of pulses (p)} \times \text{Applied electric field (E)}}$$

6.2.1 GRAPHICAL ANALYSIS

A graph from the data of Table 5.2.1 is drawn and the trend line lines are added.



Applying linear analysis,

$Y = m x + C$				
The equation would be modified for the new following standard form				
Y	represents	Final microbial count	new symbol	N_f
m	represents	Regression coefficient	new symbol	b_t
x	represents	number of pulses	new symbol	p
C	represents	Initial microbial count	new symbol	N_i

Taking natural logarithm:

$$\ln Y = -m x + \ln C$$

Rearranging and substituting:

$$\ln Y - \ln C = -m x$$

$$\ln(Y/C) = -m x$$

$$\ln\left(\frac{N_f}{N_i}\right) = -b_f E$$

[Equation 6.2.3]

$$\ln(S) = -b_t p$$

Hulsheger in 1981 developed a mathematical model for the survival rate [10], which correlates the survival ratio, with applied electric field strength and the time duration (number of pulses):

$$S = \left(\frac{t}{t_c}\right)^{\frac{-(E-E_c)}{k}}$$

[Equation 6.2.4]

Where:

S = ratio of number of microorganisms present in the food after treatment and initial number of microorganism present before the treatment,

t = treatment time, which is the product of number of pulses and pulse width (s),

t_c = critical treatment time, which is a threshold value above which inactivation occurs (s),

E = electric field strength (V/m),

E_c = critical electric field strength, which is a threshold value above which inactivation occurs (V/m), and

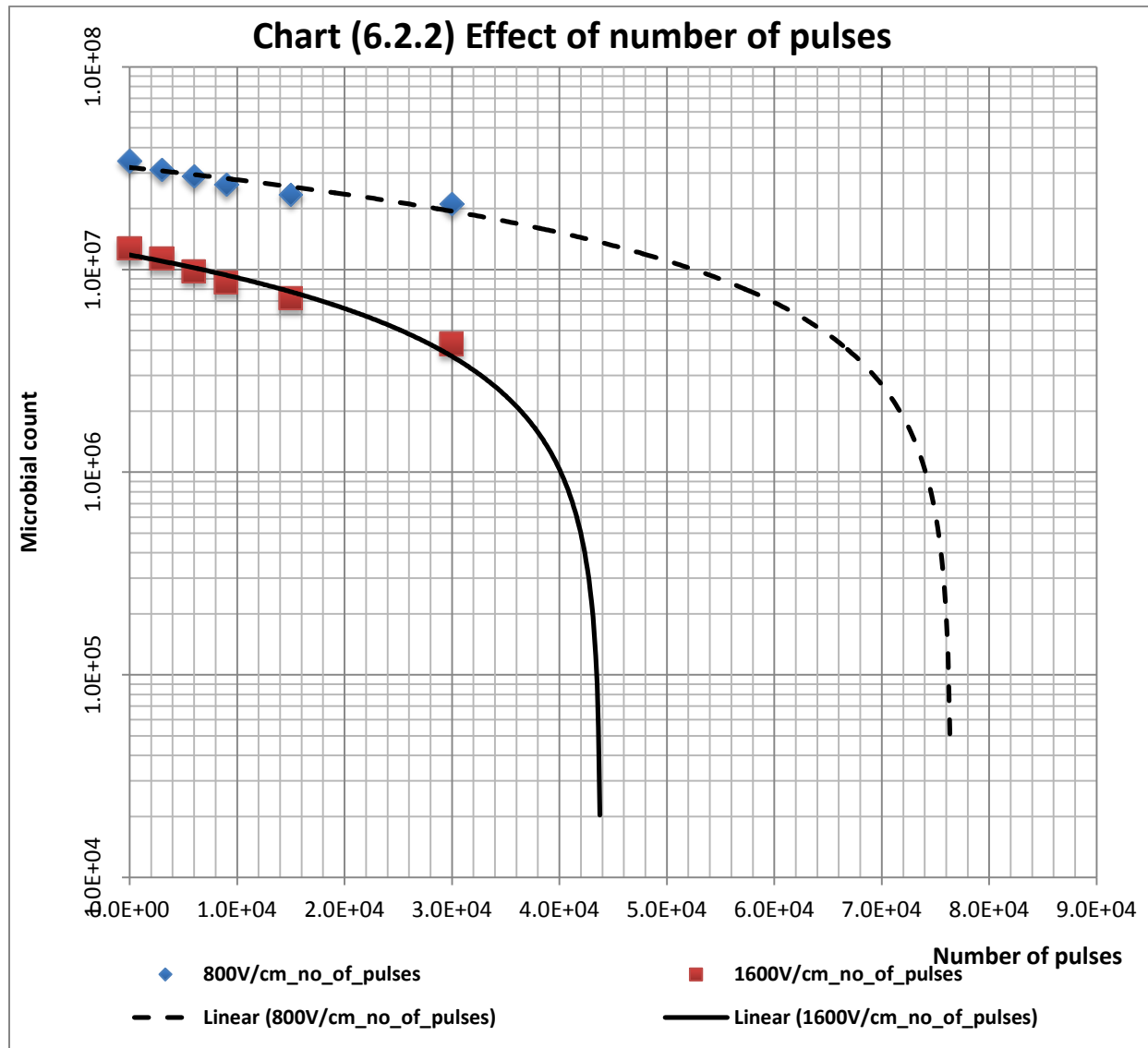
k = specific constant for a microorganism.

This equation is expressed generally with the logarithms base 10 forms by:

$$-\log(S) = \frac{(E-E_c)}{k} * \log\left(\frac{t}{t_c}\right)$$

[Equation 6.2.5]

Extending the trend line of the chart 6.2.1



From the graph, it can be concluded that for higher electric field intensity lesser number of pulses is required. Since, in this experiment, numbers of pulses are proportional to the amount of time for which samples were exposed, it can also be said that Lethality rate will increase with increasing electric field for the same amount of time.

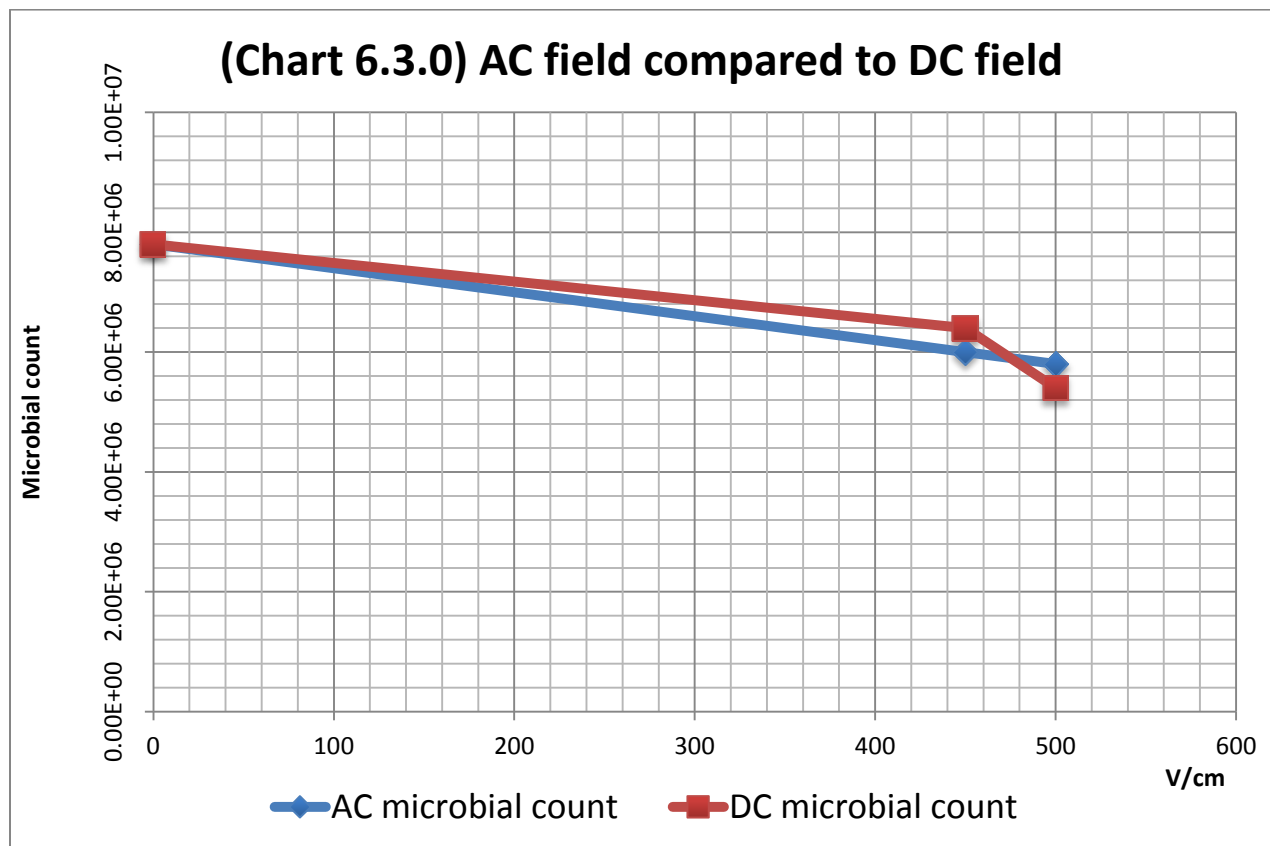
$$\text{Microbial count (N)} \propto \frac{1}{\text{Applied Electric field (E)}}$$

$$\text{Microbial count (N)} \propto \text{to the number of pulses (p)}$$

6.3 AC FIELD COMPARED WITH THE DC FIELD

The data from the Table 5.3.1 was taken, new table was made in which survival ratio was added and then the data plotted onto a graph, Chart 6.3.0

<i>Table [6.3.1] AC field versus DC field</i>		
Electric field (V/cm)	AC microbial count	DC microbial count
0	7.80E+06	7.80E+06
450	6.00E+06	6.40E+06
500	5.80E+06	5.40E+06
Survival ratio	0.74	0.69



From the above graph, it can be stated that, at low voltages, there is no difference between both AC and DC fields. This is further confirmed by the closeness of the Survival ratio of the microorganisms.

6.4 DATA SHOWING THE RELATIONSHIP BETWEEN OF THE THICKNESS OF THE PLATES AND PLATE SEPARATION DISTANCE TO THE COLONY AND THE MICROBIL COUNT

In the design of the test chamber it is important to note the co-relationship between the thickness of the electric plates and the distance between the plates where the test sample is placed. Table 6.4.0 illustrates the following and a corresponding graph Chart 6.4.0 further justifies the design parameters.

Table [6.3.2] Thickness of the plates				
Electric field (V/cm)	Distance between the plates (mm)	Thickness of the plates (mm)	Ratio of separation	Microbial count
0	5	1	0.2	2.84E+08
950	5	1	0.2	2.68E+08
950	5	2	0.4	1.89E+08
950	5	6	1.2	1.34E+08
950	5	10	2.0	9.30E+07
950	5	12	2.4	7.50E+07
950	5	14	2.8	4.50E+07
950	5	16	3.2	3.20E+07
Survival ratio				0.11

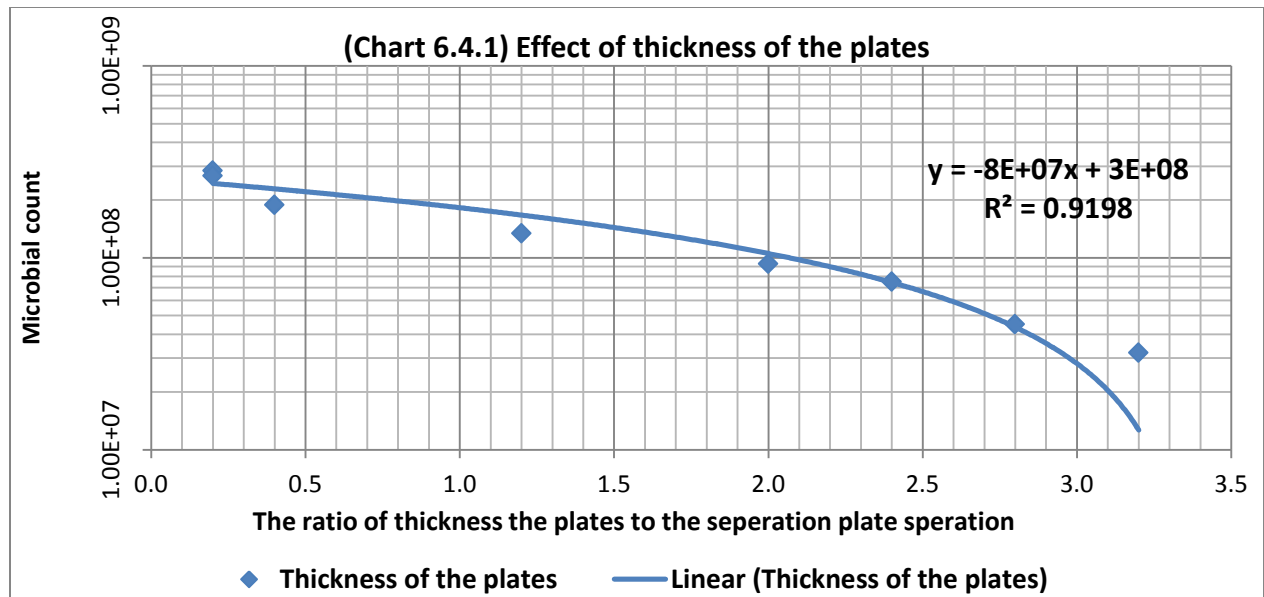
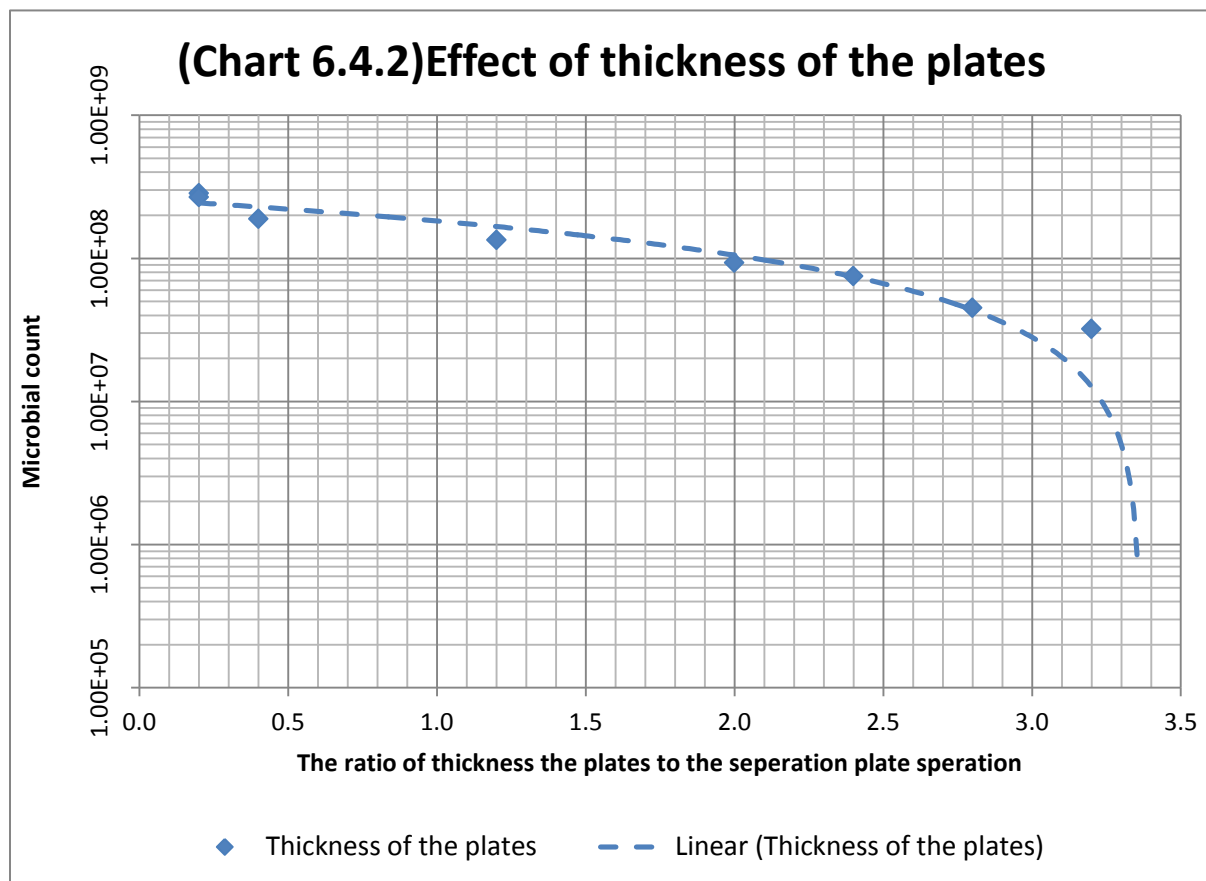


Chart 6.4.2 shows the optimum design parameter for 5:16, separation to thickness ratio. With the increasing thickness, Survival ratio decreases, but only up to a certain extent.



It can be said that:

$$\text{Microbial count (C)} \propto \text{Seperation ratio (S)}$$

CHAPTER 7: DESIGNING AND MODELLING OF THE TEST CHAMBERS

7.1 Design of the test chamber

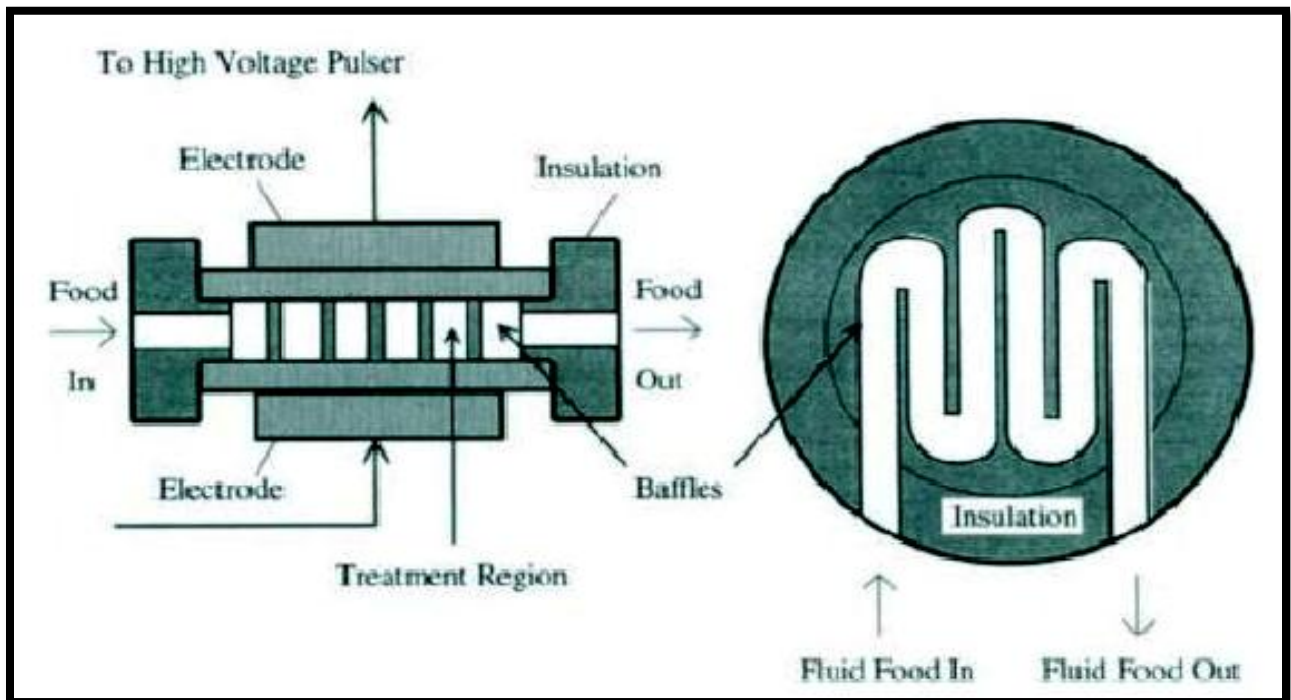
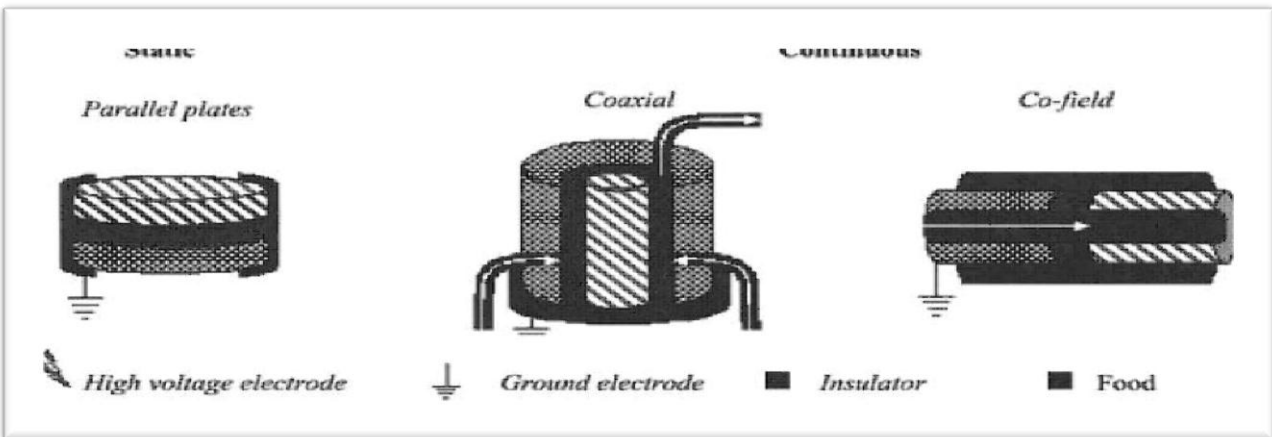
One of the significant and intricate apparatus of any PEF processing system is the designing of the treatment chamber. Its function is only to transfer the generated pulsed electric fields to the food sample, but also at the same time preferably to obtain a reasonably high electric field intensity without any loss. An ideal treatment chamber design would ensure that all of the food samples are equally exposed to the same electric field intensity, the number of pulses and the temperature.

Generally, the static treatment chamber consists of two electrodes or plates held in position by insulating materials which also helps to enclose or to hold the food samples. However, a wide range of designs both of static and continuous chambers have been suggested by various authors [1]. The first systems were static and were designed for the treatment of small volumes of samples. Many of these have parallel electrodes of stainless steel or carbon, imbedded in different kinds of materials, with spacers made of different kinds of insulators: polyvinyl, nylon, glass, fiber glass etc. The use of different spacers, made it possible to adjust the distance between electrodes and hence the strength of the applied electric field.

The following factors are both crucial and important when designing a PEF Treatment Chamber [3]

- Electrical breakdown in liquid foods is very sensitive to local enhanced electric fields within the chamber. By designing a proper chamber, electric field enhancement points can be located outside of the treatment region.
- Washable and autoclavable (sterilize-able) materials must be used in constructing a treatment chamber. Polysulfide and stainless steel materials are recommended as insulation and electrode material, respectively.

- Filling and removal port must be easily accessible. Gas bubbles can become trigger sites for dielectric breakdown. Therefore, the filling port must facilitate expelling air during filling.
- When repeatedly applying high electric field, the energy input must be considered. Cooling of the electrodes is required to maintain low temperature operation, which can be done by circulating water through a jacket built onto the electrodes.



7.2 PRACTIAL STATIC CHAMBER, AS CONSTRUCTED FOR THE EXPERIMENT

For experimental use, the static test chamber was built using:

- a) Glass casing (2x)
- b) Steel plates 10 mm thick (2x)
- c) Aluminum covering
- d) Separation distance of 5 mm
- e) Adhesive glue
- f) Laminating layer



Figure 7.1.a Prototype of the two chamber “plates”

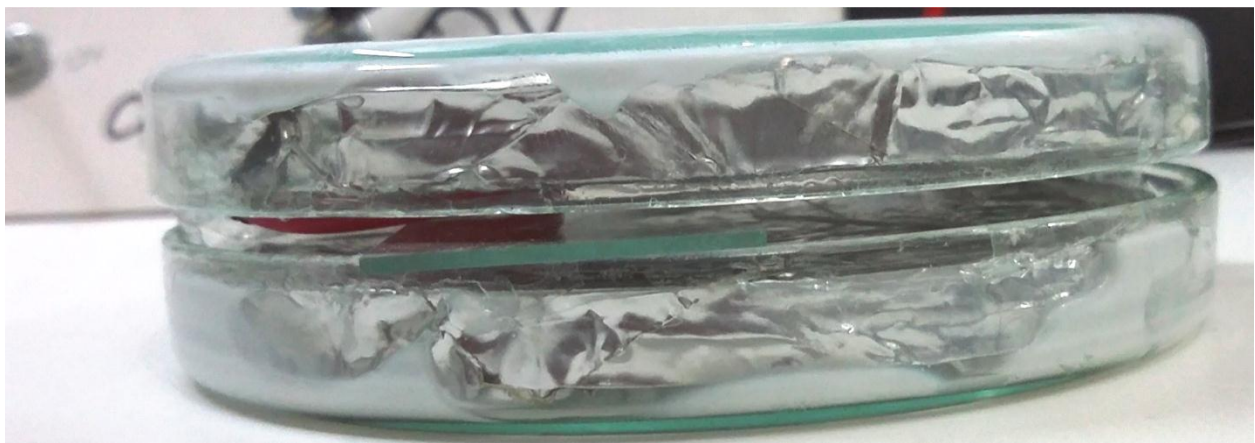


Figure 7.1.b Prototype of static chamber used while conducting experiments

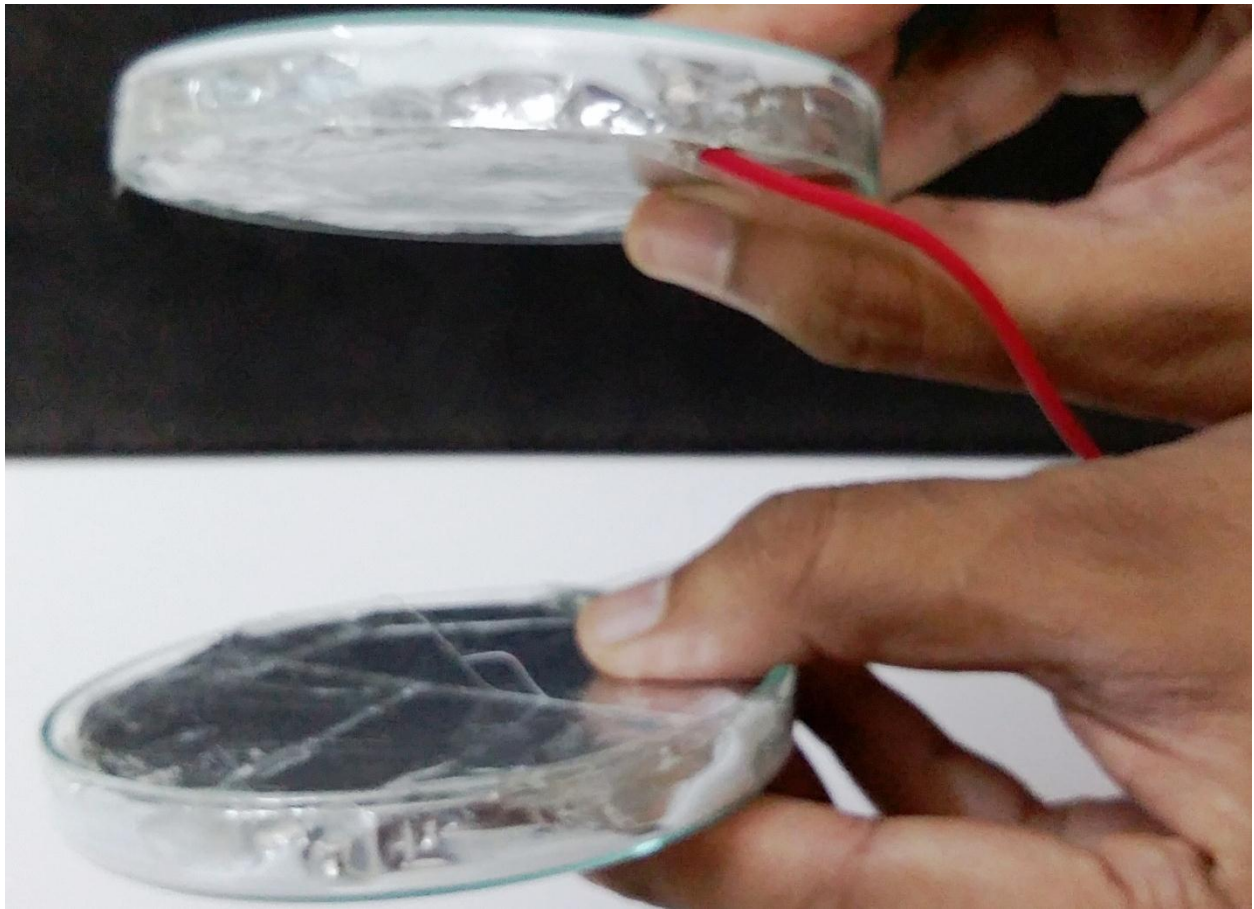


Figure 7.1.c the horizontal view of the prototype



Figure 7.1.d the top view of the prototype

Chapter 8: Conclusion and future works

The PEF was able to neutralize microorganisms. The Lethality Rate was directly proportional to the applied electric field. Raw milk samples treated at 1.60kV/cm showed an average microbial survival rate of about 21%, for fresh mango juice it was 54.3%, for sugarcane extract it was 36.4% and for tap water the rate was 47.5%. All samples showed an average microbial survival rate of less than 1%, when the applied electric field's data was extrapolated to 3.3kV/cm. The number of pulses also effected the microbial growth; general the Lethality Rate was directly proportional to the number of pulses. It was also found that same number of pulses applied at 1.60kV/cm was more effective than at 0.8kV/cm. At low voltages, there is no difference either between both AC and DC electric fields. This was confirmed by the closeness of the Survival ratio of the microorganisms. And finally, ratio of the thickness of the plates of the test chamber to the distance between the plates was found to play an important role. Survival ratio of the microorganism was inversely proportional to the ratio of separation. The main advantages of the PEF technology are that it can neutralize vegetative cells. The food colors, flavors, taste, texture, nutritional values are preserved giving the food a "natural" taste. There are no evidences of toxicity and food items are treated relatively for short periods of time as compared to other processes. The process is best suited for of heat sensitive edible foods items: specially liquid and semi-liquid foods and also for pasteurization of milk, fruit juices, soups, purees and alcoholic beverages, amongst others. The technology also is environment friendly.

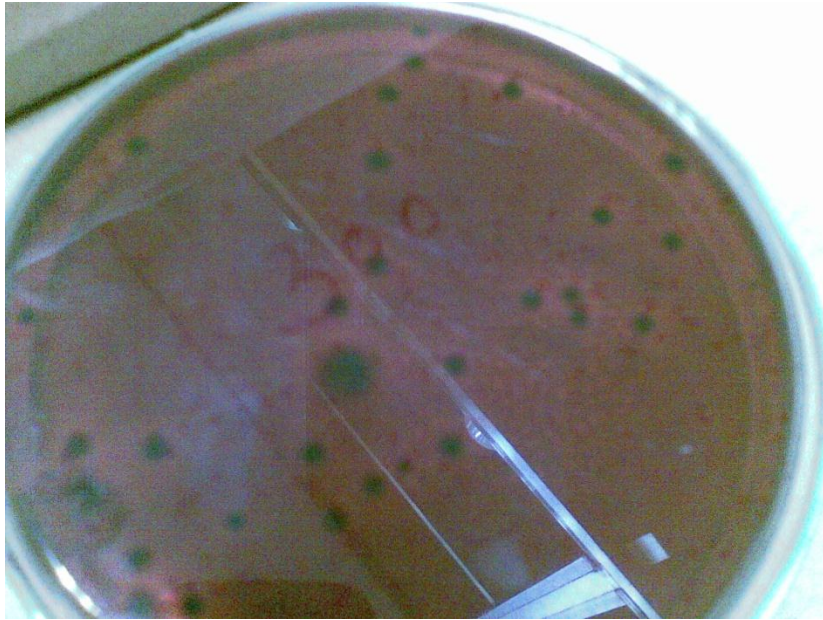
There are many aspects of the PEF technology which are not completely explored; therefore the field provides an extensive area for research and for future developments. Scientists and researchers from around the world are focusing on various aspects of this technology; one of more relevance is to implement the technology from laboratory level to commercial and industrial scale. The impact of processing conditions such as temperature, pH, moisture, protein, lipid and ions contents on the safety and quality of the test products are yet to be studied and researched. Despite these barriers this technology has the incredible potential to replace or compliment conventional methods of the food processing industry.

REFERENCES:

- [1] G.V.Barbosa,Canovas,U.R Pothakamury, M. M.Gongora-Nieto and B.G.Swanson, “Preservation of Foods with Pulsed Electric Fields”,San,1999. 4(15): 142-153
- [2] Ho. S, and Mittal. G.S, “Electroporation of cell membranes”, 1996. 16(4): 349-362.
- [3] Qin, B.L, Barbosa-Cánovas, G.V, Swanson, B.G. and Pedrow P.D, “Inactivation of microorganisms by pulsed electric field of different voltage waveforms”, 1994. 1(6): 1047-1057.
- [4] Mertens B. and Knorr D, “Development of non-thermal electrical pasteurization food Technology”, 1992. 5(16): 124-133
- [5] Calderón-Miranda, M.L, Barbosa-Cánovas, G.V. and Swanson, B.G, “Transmission electron microscopy of *Listeria innocua* treated by pulsed electric fields and nisin skimmed milk. International Journal of Food Microbiology”, 1999a. 51(8): 31-38
- [6] Ali Mohammad Dastgheib, Zolkafle Buntat AND S.M. Zafar Iqbal, “Effect of High Voltage Electric field (AC & Impulse) on Fresh Pineapple”, 2012. 8(16): 59-68
- [7] Hülshager, H. Potel, and Niemann, E.G, “Killing of bacteria with electric pulses of high field strength. Radiation & Environmental Biophysics”, 1981; 5(18):224-232
- [8] Zhang, Q.H, Qin, B.L, Barbosa-Cánovas, G.V. and Swanson, B.G, (1995b). “Inactivation of *Escherichia coli* for food pasteurization by high-strength pulsed electric fields”, 1995b. 19: 103-118
- [9] Reitler W, “Conductive heating of foods. Munich”, 1990. 17(32): 204-210
- [10] Malek Amiali, “Inactivation of *Escherichia coli* O157:H7 and *Salmonella Enteritidis* in Liquid Egg Products using Pulsed Electric Field”, 2005. 12(23): 253-264
- [11] Yashwant Kumar, Krishna Kumar Patel and Vivek Kumar, “Pulsed Electric Field Processing in Food Technology”, 2015. 33(54): 354-364

- [12] J. McHugh, S. Abiteboul, R. Goldman, D. Quass and J. Widom, “A Database Management System for Semistructured Data”, 1997. 5(15): 73-83
- [13] Bailey, W. R and E. G. Scott, “Diagnostic microbiology: a text book for the isolation and identification of pathogenic microorganisms”, 1966. 4(32):67-74
- [14] Brenner, Egon. Javid, Mansour, "Symbols and Polarity of Mutual Inductance in Chapter 18 – Circuits with Magnetic Circuits”, 1959. 3(34):33-45
- [15] MacFaddin JF, “Media for isolation-cultivation-identification-maintenance of medical bacteria”, 1985. 35(24): 253-264
- [16] Prough, D. S. and Bidani A, “ Anesthesiology”, 1999. 3(54):343-354
- [17] Booth, C. et al, “ *Extremophiles*”, 2006. 5(34): 343-354
- [18] Goldman, Emanuel Green, Lorrence H, “Practical Handbook of Microbiology, Second Edition (Google eBook)”, 2008. 36(5): 63-78
- [19] Visvesvara GS, Pathogenic and Opportunistic Amebae, “Manual of Clinical Microbiology”, 2007. 2(34):543-557

APPENDIX

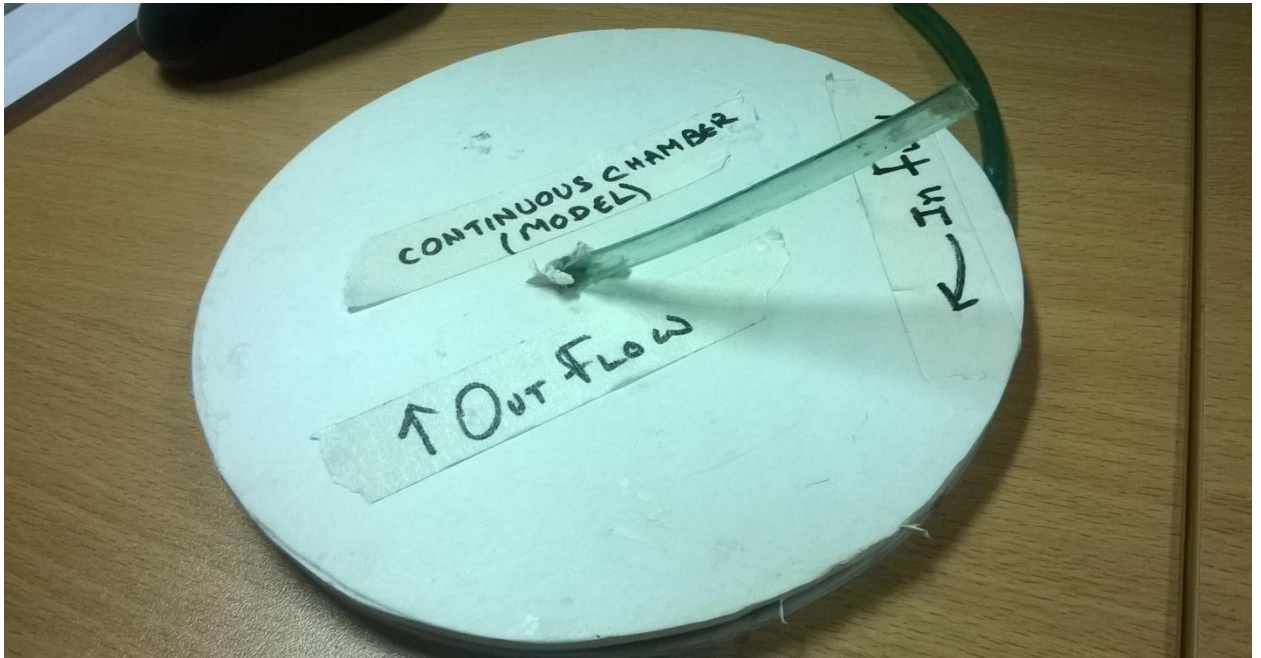


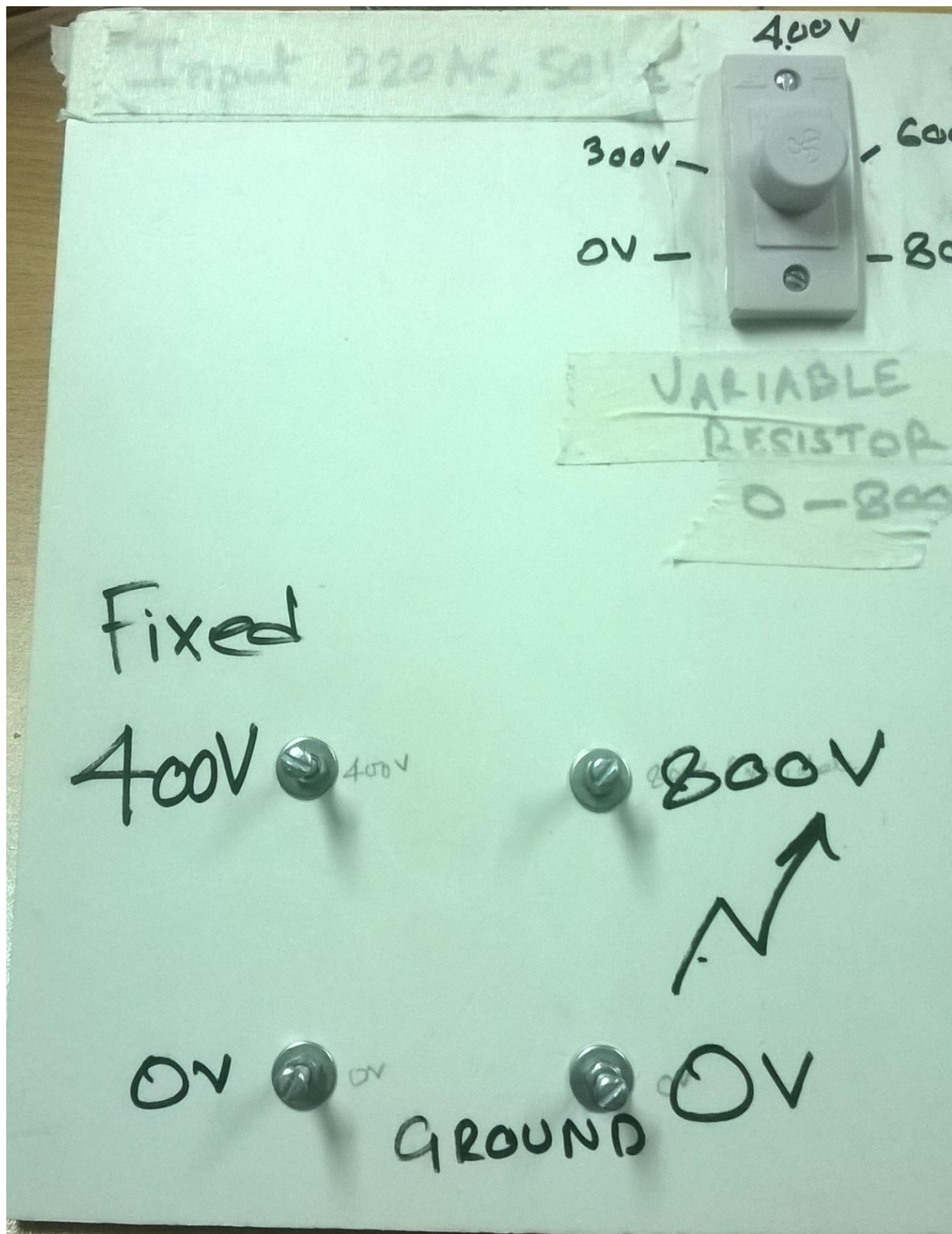
Petri dish showing colony growth (300 counts)



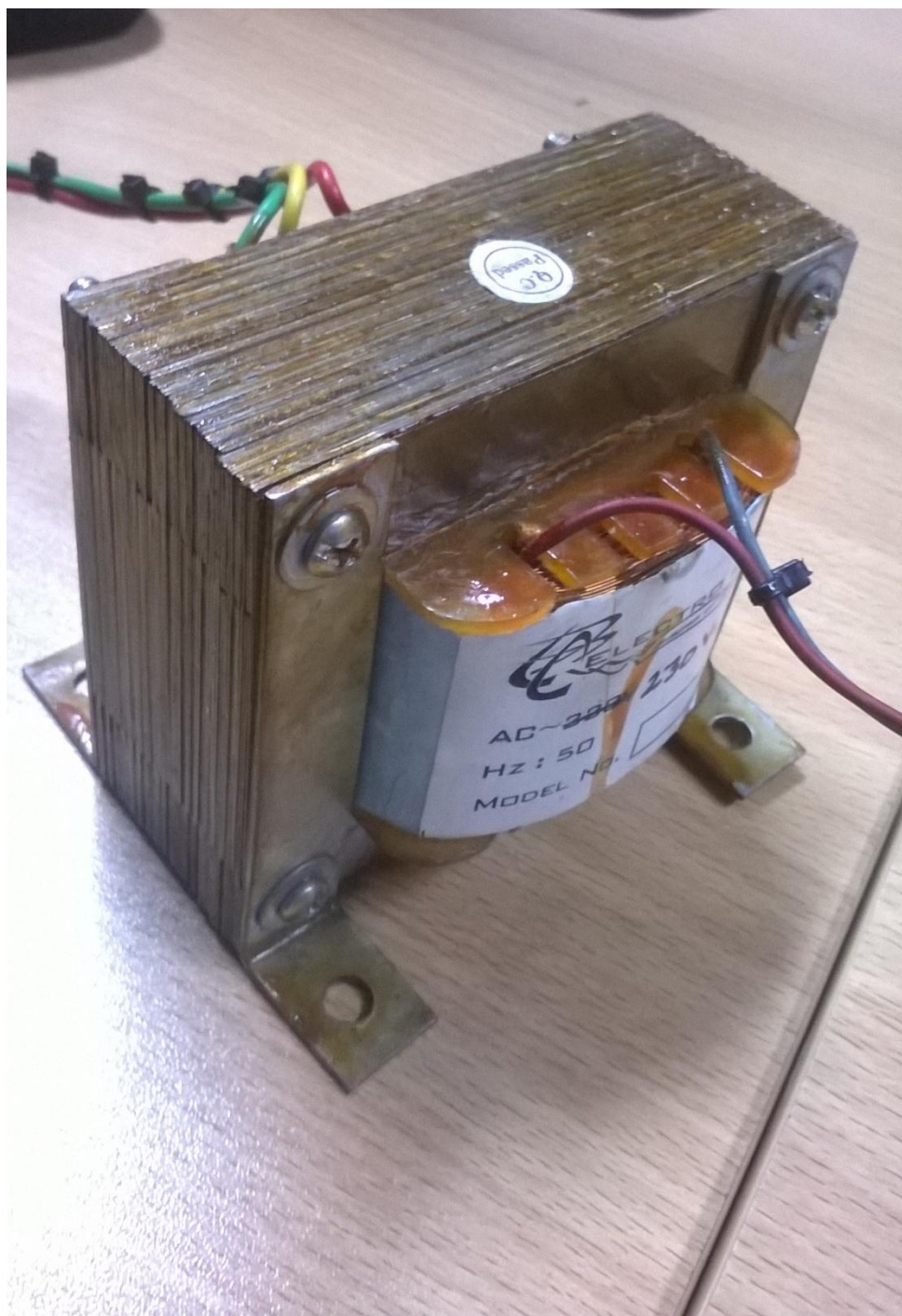
Petri dish showing colony growth (over count)

A model of a continuous treatment chamber





Front view of the supply unit



Custom- built transformer

Serial dilution and sample preparation

