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Modeling of Electroporation and Electric Field Investigation for a Single Cell Dispersed in Liquid Foods

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Abstract

Inactivation of microorganism by Pulsed Electric Field (PEF) is an important research topic because of its potential as an alternative to thermal pasteurization methods. Applying the pulsed electric field technology in food preservation is a promising non thermal method and provides consumers with microbiologically safe, minimally processed and increases the shelf life of liquid foods. This paper aims to study the effects of electric field intensity on *E. Coli* cell with different shapes such as ellipsoidal and spherical shape suspended in a conducting medium (milk) using the simulation package MAXWELL. The electric field analysis of bacteria (*E. coli*) with and without preexisting pore was carried out for uniform and non uniform field configuration and the induced transmembrane potential was calculated analytically and numerically

Keywords: Pulsed Electric Fields (PEF), Sterilization, Transmembrane Potential (TMP)

1. Introduction

The application of electric field to biological cells suspended in a medium cause's buildup of electric charges at the cell membrane. An external electric field can induce an electric potential difference across cell membrane called as Transmembrane Potential (TMP). If the induce transmembrane potential[1] is large enough i.e. above threshold value of 1V, the cell membrane becomes permeabilized in a reversible process called electro permeabilization, thus allowing entrance of molecules easily across cell membrane. Further increase of an electric field causes irreversible membrane permeabilization and cell death. Therefore investigation of induced potential distribution[2,7] on the cell membrane is important in studying the effects of electric fields on biological cells. Potential distribution on the surface of cell[3] placed in an electric field can be calculated numerically using Finite Element Method. Thus the critical electric field intensity is analyzed for different factors such as applied voltage and the electrode geometries. A static PEF treatment chamber unit with parallel plate electrodes to test the feasibility of pasteurizing foods with pulsed electric field. A model for a single spherical cell electroporated by the application[4] of an electric field and also investigated the effect of resting potential on the transmembrane potential for an electroporated cell. The application of outer membrane electroporation process in various fields such as biofouling[5,6] prevention, drug delivery for cancer treatment and bacterial decontamination in liquid foods. The opening of a pore exist in the cell membrane was mainly due to the force generated by the application of an electric field. The force generated depends on the difference in dielectric constant between the fluid and the membrane. The analytical method for the description of the transmembrane voltage induced on arbitrarily oriented ellipsoidal cells under the assumption of non-conductive membrane. The different factors influencing the electromechanical breakdown of the cell membrane during the sterilization of liquid food using pulsed electric field technology[8,9]. The dielectric rupture theory and electroporation theory involving in breakdown of the cell membrane.

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2. Mechanism of Electroporation

Electroporation (EP) explained as a phenomenon in which a cell exposed to the high voltage electric field that temporarily destabilizes the Bilayer Lipid Membrane (BLM) and protein channels of the cell membrane (Castro). The opening and closing of protein channel is dependent on TMP. The gating potential of the channels constituted by proteins is in the range of 50 mV. When PEF[10,11] is applied, the voltage sensitive protein channels will open, experience current much larger than the current normally experienced during metabolic activities and as a result protein channels are irreversibly denatured (i.e. eliminates enzyme activities of the protein channels). The lipid bilayer is also susceptible to applied electric fields due to its net electric charge. The application of electric field causes lipid molecules to reorient[12] thus creating hydrophilic pores which conducts current and eventually leads to the rupture of cell wall. Thus electroporation in the cell membrane occurs both in protein channels and in the lipid bilayers resulting in the inactivation of cell.

During electroporation, Lipid Bilayer suffers from any one of the following possible fates on the membrane namely,

1. A slight increase in membrane conductance.
2. Mechanical rupture of the cell wall or any structural changes in the membrane.
3. Reversible electrical breakdown resulting in complete discharge of the membrane.
4. Irreversible electrical breakdown when the intensity of the electric field is raised.

3. Finite Element Analysis

Numerical method is the one of the best method that can realize the results more accurately for complex cell geometries when compared to analytical methods. This method divides the problem domain area in to smaller number of finite elements and requires a lesser computation time compared to analytical approach. A finite element model of a cell in a conductive medium was build using the software package Maxwell 2D simulator (ANSOFT). To analyze a problem we should specify the appropriate geometry, boundary conditions, material property of the treatment chamber and excitation of device by means of applying an input voltage in kilovolts.

1. Problem Statement
   To analyze the effect of electric field strength on a biological cell (*E. coli*) with different shapes such as spherical and Ellipsoidal shape suspended in a conducting medium (milk) using finite element method.
2. Domain
   This is an interior problem since the field problem domain is enclosed by a balloon boundary. All space outside this boundary is excluded from the field problem domain.
3. Type of Problem
   Biological cells are usually negligibly magnetizable. Hence it is an Electrostatic problem.
4. Dimension
   For electrostatic modeling, the insulating plates represents the mirroring plane boundary condition and thus the field distribution on the membrane are the same for the whole spheroid cells as for half of the cell placed on the insulating plate and thus the problem becomes two-dimensional problem.
5. Medium
   Here inside the cell, dielectric medium is Isotropic (i.e., it has the same behavior with respect to a field at a point regardless of the direction of that field), uniform with relative permittivity.
6. Governing Equation
   The governing equation for this problem can be obtained by solving Laplace equation $\Delta^2 \Phi = 0$. (By neglecting the space charge effects)
7. Material Properties
   The following are the dielectric constant and conductivity values of the bacterial cell suspended in the conductive medium (milk).

Inner Membrane	: $\epsilon r = 80$	$\sigma = 1.0$ (S/m)
Outer Membrane	: $\epsilon r = 2.5$	$\sigma = 1e\text{-}5$ (S/m)
Pore	: $\epsilon r = 80$	$\sigma = 0.1$ (S/m)
Medium (Milk)	: $\epsilon r = 69.5$	$\sigma = 0.5$ (S/m)

4. Model of the Treatment Chamber

The treatment chamber is made up of borosilicate glass material which is compact in size and is enclosed by a Teflon insulator at the two corners of the chamber. Two electrodes are placed at both the side of the chamber which is movable for various distances in millimeters. A testing fluid (milk) is poured inside the enclosed chamber where the modeled bacterium of different shapes such as ellipsoidal and spherical shape was placed in between the electrode. The width and length of the treatment chamber is 22 mm and 130mm and the electrode gap spacing is about 5mm. Figure 1 shows the model of the treatment chamber with uniform field electrode configuration.

The finite element analysis was carried for two different electrode geometries and the effect of electric field intensity for the applied voltage of 1 kV was analyzed for the treatment chamber without bacteria.

From the Figure 2 and 3, finite element analysis investigates the electric field intensity as a function of distance under uniform and non uniform field. It is observed, at the High Voltage (HV) electrode surface the value of electric field intensity is maximum and it goes on decreasing when the distance between the electrodes increases. From the Figure 4 and 5 shows the potential distribution inside the treatment chamber without bacteria under uniform and non uniform field configuration. It is inferred, at the High Voltage (HV) electrode surface the potential

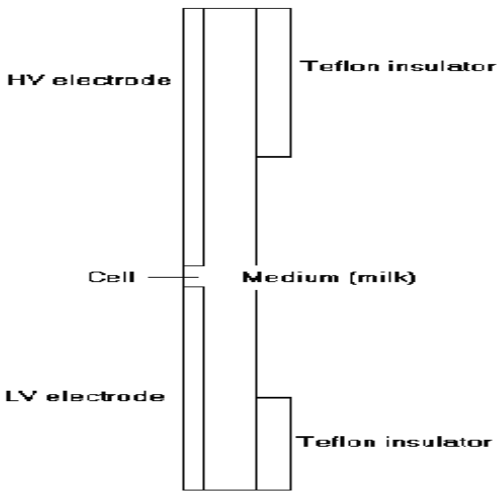


Figure 1. Model of the Treatment Chamber.

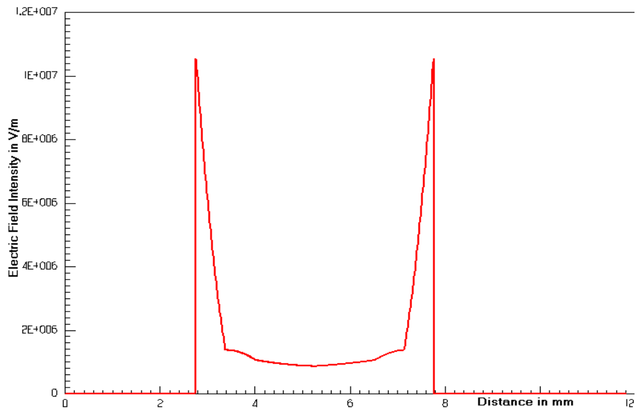


Figure 3. Field plot for treatment chamber without bacteria in nonuniform field.

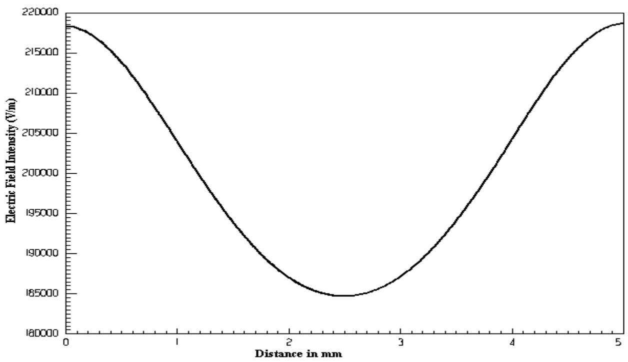


Figure 2. Field plot for the treatment chamber without bacteria in uniform field.

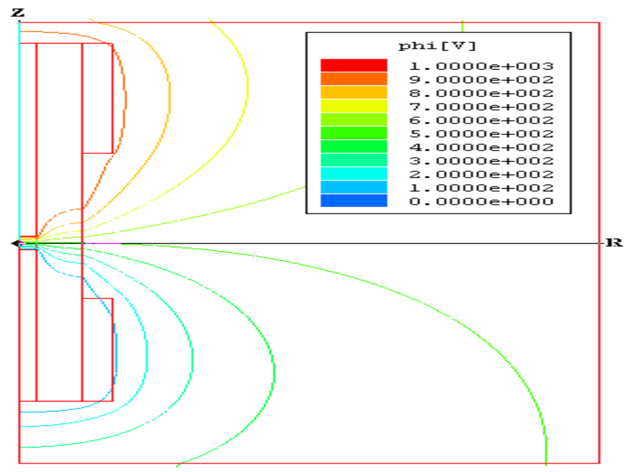


Figure 4. Equipotential plot for the treatment chamber with out bacteria under uniform field.

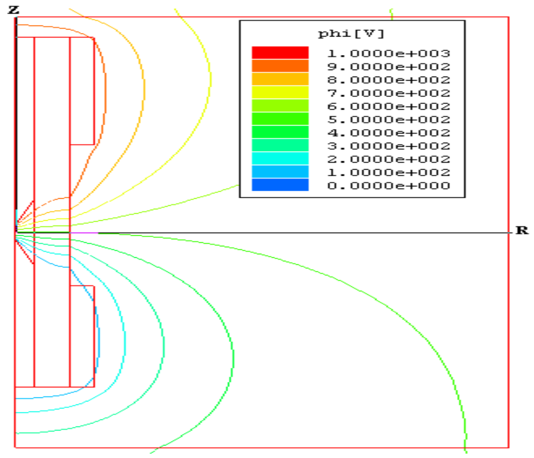


Figure 5. Equipotential for the treatment chamber without bacteria under non uniform field.

is maximum and it goes on decreasing when the distance between the electrodes.

4.1 Mathematical Model of Cell with Pore

A simple bacterium model showing the three regions namely cytoplasm, inner and outer membrane are not suffient to understand the mechanism of the electroporation. Hence a mathematical model of the cell with pore is required to analyze the effect of electroporation[13] during the inactivation mechanism. The following Figure 6 and 7 shows the mathematical model of the *E. coli* cell considered to have spherical and ellipsoidal shape with pre-existing pore on the membrane.

Inactivation of microbes is related to the electromechanical instability of the cell membrane. A pore which

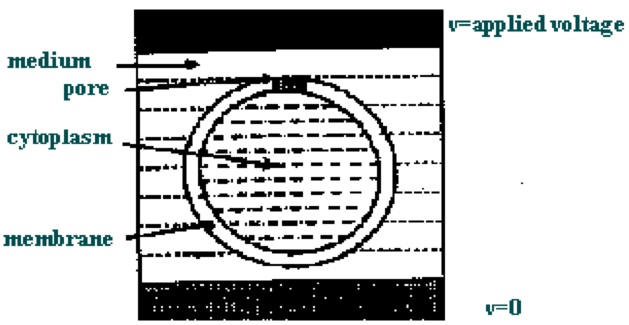


Figure 6. Model of Spherical shape *E. coli* cell with pore.

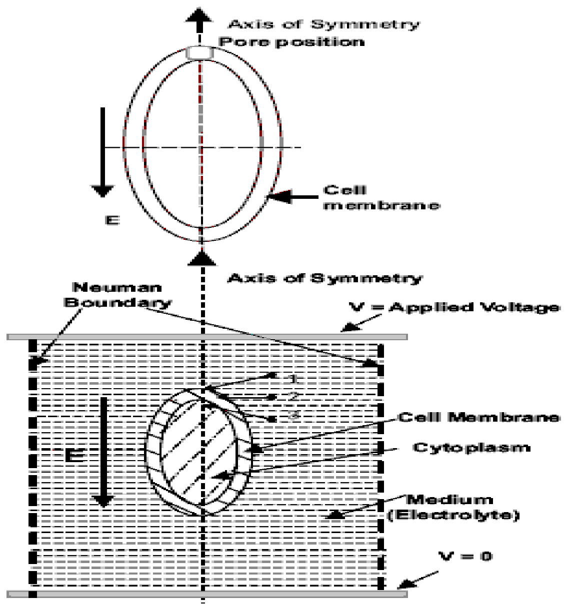


Figure 7. Model of Ellipsoidal shape *E. coli* cell with pore.

occurs naturally in the cell membrane will be sealed before the PEF treatment. But after the application of high voltage pulses, electric field generates a force which tends to open or unseal the pore known as electroporation mechanism. The force generated depends on the electric field in the pore and the difference in dielectric constant between the membrane and pore.

The effects of electric field intensity on the cell shape such as spherical and ellipsoidal shape were analyzed using finite element method. Also it was carried out for two different electrode geometries showing uniform and non-uniform field conditions by taking different cases such as

1. Treatment chamber containing the bacteria cell without pore on its membrane.
2. Treatment chamber containing the bacteria cell with pore on its membrane.

4.2 Field Distribution in the Cell Membrane

4.2.1 Ellipsoidal Cell

The electric field intensity required to produce TMP of 1V leading to cell wall rupture has to be determined. The finite element analysis was carried out by taking two cases such as bacterial cell with and without pore on its membrane. The major axis radius of 10 μm, minor axis radius of 5 μm and the cell membrane thickness of 1μm are the dimensions of the ellipsoidal bacterial cell. In this section FEM analysis was carried out for the ellipsoidal cell without pore on its membrane to study the electroporation process which occurs during the mechanism of microbial inactivation. The Figure 8 shows the field plot for the bacterial cell without pore on its membrane as a function of distance which clearly states the occurrence of breakdown of the cell membrane at about 2.5 mm under uniform field.

Different electrode configuration have a considerable effects on the electric field intensity field and it is the most important processing factors which strongly influence microbial inactivation during the sterilization of liquid food samples by pulsed electric field. Higher the electric field intensity higher will be the inactivation rate of the microorganisms suspended in the liquid food and it is achieved in the non uniform field configuration which is as shown in Figure 9.

Electroporation which occurs in two steps such as formation of a pore (if not already present) and opening of the pore. In real cell membranes, pores are normally present. For model membranes on which many studies are carried out without considering preexisting pore on the membrane. To understand the actual mechanism of electroporation during the sterilization process, an analysis was done by considering the presence of pore on the cell membrane[14]. After pore formation the pore conductance increases which leads to the breakdown of the cell membrane. The Figure 10 shows the field distribution for the treatment chamber containing the bacterial cell with pore on its membrane under uniform field configuration.

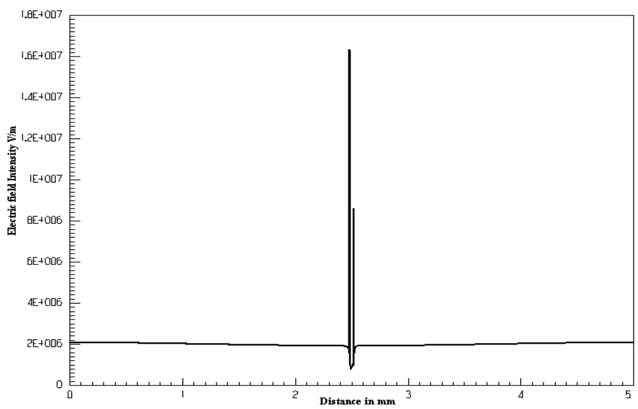


Figure 8. Field Plot for bacterial cell without Pore under uniform field.

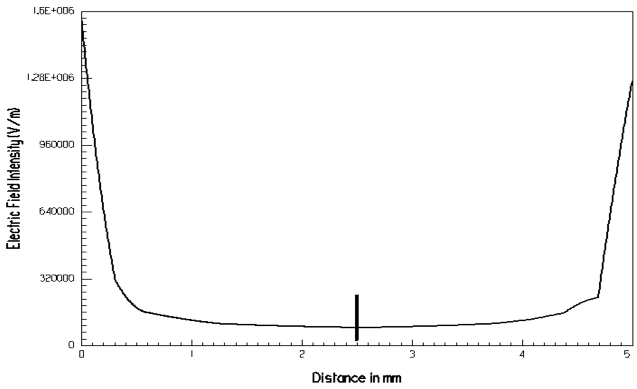


Figure 9. Field Plot for bacterial cell without Pore under non uniform field.

4.2.2 Spherical Cell

The spherical cell with different cases such as with and without preexisting pore on its membrane were analyzed using the finite element method in order to ensure the role of cell size and shape during the mechanism of electroporation involved in the sterilization of liquid foods using high voltage pulses. The spherical cell with a radius of 10 μm and the cell membrane thickness of 1μm were taken for the analysis under uniform field. The FEM analysis was carried out for the spherical cell without pore on its membrane to study the electroporation process which occurs during the mechanism of microbial inactivation. From this analysis, the parameters such as electric field intensity and induced transmembrane potential are calculated numerically.

The Figure 11 and 12 illustrates the field plot for the bacterial cell without pore on its membrane as a function of distance under uniform and non uniform field configuration. It is inferred that the desired electric field strength produced under the non uniform field is very high compared to the uniform field for the same applied voltage. Hence the inactivation is increases proportionally with respect to the electric field strength.

A pore which occurs naturally in the cell membrane will be sealed before the PEF treatment. But after the application of high voltage pulses, electric field generates a force which tends to open or unseal the pore known as electroporation mechanism. The force generated depends on the electric field in the pore and the difference in dielectric constant between the membrane and pore[15]. At the high voltage electrode surface the potential distribution is maximum and it goes decreasing when the

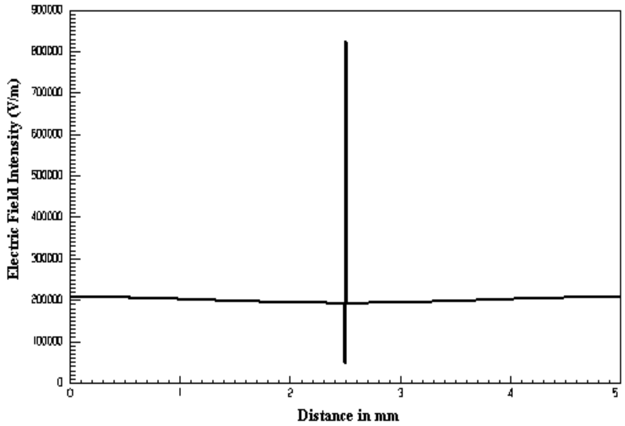


Figure 10. Field plot for bacteria cell with pore under uniform field.

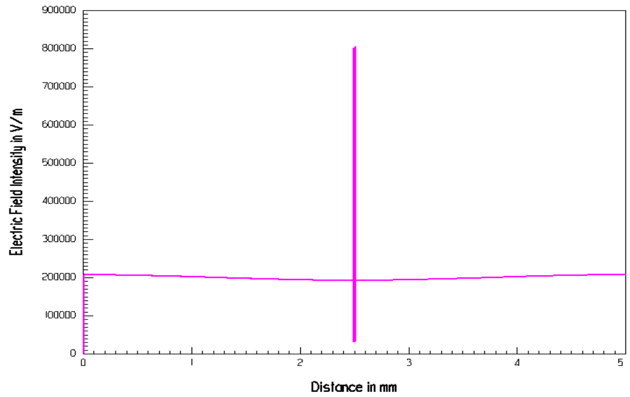


Figure 11. Field plot for bacteria without pore under uniform field.

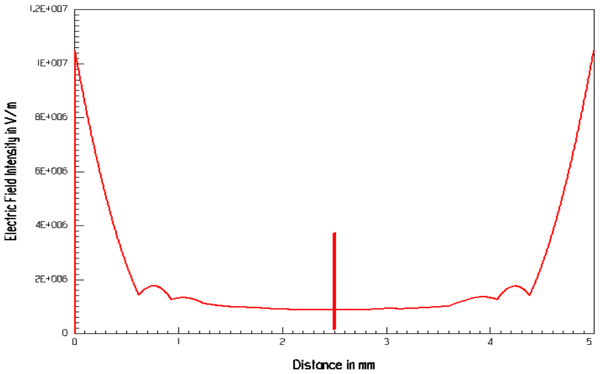


Figure 12. Field plot for bacteria without pore under non uniform field.

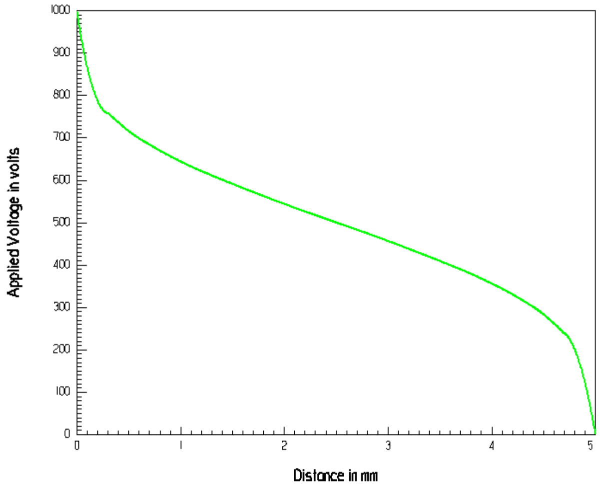


Figure 13. Potential plot for the treatment chamber with Bacteria.

distance between the electrodes increases is shown in the Figure 13.

Figure 14 and 15 illustrates that the field plot of the cell with pore on its membrane under uniform and non-uniform fields.

For the ellipsoidal cell and spherical the field in the cell membrane is caused largely by the relaxation of field in the cytoplasm due to the difference in dielectric constants of the cytoplasm and cell membrane. Figure 16 and 17 illustrates that the ellipsoidal cell without pore on its membrane requires lesser electric field intensity compared to spherical cell to produce TMP of 1V which leads to the rupture of cell wall under uniform field configuration.

The larger *E. coli* cells are less sensitive to lower electric field pulses. So it requires higher field intensity for the rupture of cell wall which is proved from the FEM analysis. Under uniform field, ellipsoidal cell without pore on its membrane under uniform field requires average electric field strength of about 3 kV/cm where as spherical cell without pore on its membrane requires average electric field strength of about 7 kV/cm to produce a threshold value of 1V TMP essential for the inactivation mechanism.

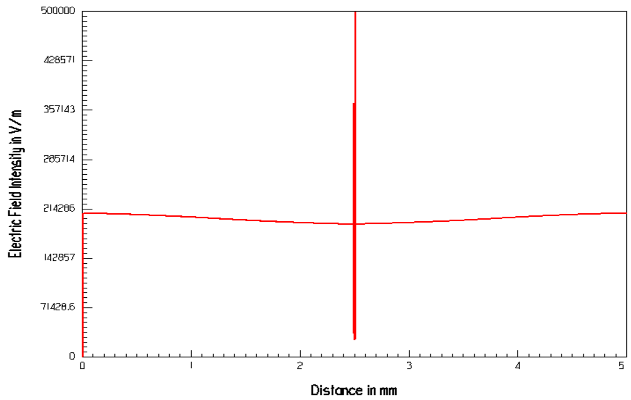


Figure 14. Field plot for bacteria with Pore under uniform field.

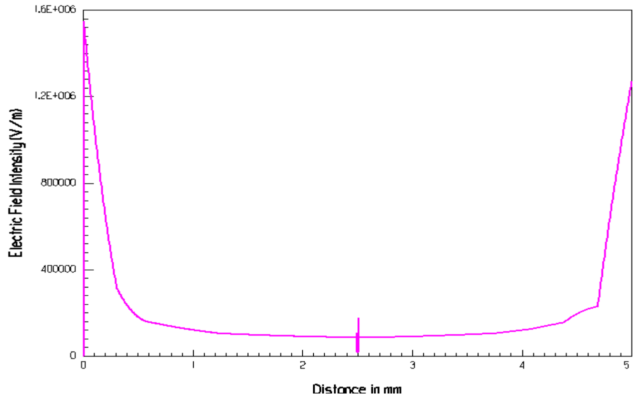


Figure 15. Field plot for the bacteria with pore under non uniform field.

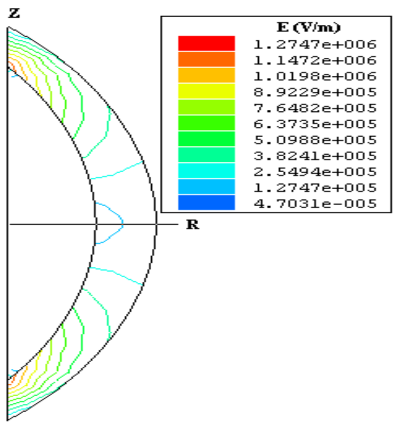


Figure 16. Field distribution in the ellipsoidal cell membrane.

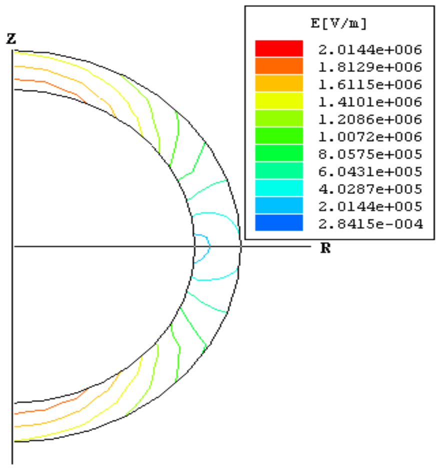


Figure 17. Field distribution in the Spherical cell membrane.

5. Evaluation of Transmembrane Potential

In this study, effects of electric field intensity for the bacterial cell with and without pore on its membrane for two electrode configuration was analyzed bacteria were analyzed using finite element method and the results are tabulated. Comparing the Table 1 and 2, the results shows that the ellipsoidal bacteria with preexisting pore requires a lesser electric field intensity of about 3 kV/cm under uniform field to produce the trans membrane potential of 1V under necessary for the rupture the cell membrane during the inactivation of microorganisms whereas spherical

Table 1. TMP for ellipsoidal cell under uniform field

Applied voltage in kV	Electric Field Intensity (E) in kV/cm	TMP for Bacteria without pore in volts	TMP for bacteria with pore in volts
0.5	1	0.5431	0.6125
1.0	2	0.9148	0.9943
1.5	3	0.9543	**1.1242**
2.0	4	**1.0754**	1.1703
2.5	5	1.0921	1.2872
3.0	6	1.1672	1.4342
3.5	7	1.3827	1.8593
4.0	8	1.6542	1.9047
4.5	9	1.8723	2.1703
5.0	10	1.9730	2.4531

Table 2. TMP for spherical cell under uniform field

Applied voltage (V) in kV	Electric Field Intensity (E) in kV/cm	TMP for Bacteria without pore in volts	TMP for bacteria with pore in volts
0.5	1	0.1439	0.4431
1.0	2	0.2859	0.6125
1.5	3	0.4281	0.9043
2.0	4	0.5732	**1.1703**
2.5	5	0.7148	1.2872
3.0	6	0.8641	1.4342
3.5	7	**1.0007**	1.8593
4.0	8	1.1442	1.9047
4.5	9	1.2872	2.1703
5.0	10	1.4302	2.4531

cell requires field intensity of 4 kV/cm. Hence the smaller cells require lesser field intensity for the breakdown of cell membrane.

Figure 18 and 19 shows the variation of TMP under uniform field for ellipsoidal and spherical cell. By analytical Method, for the orientation angle of the cell α=90°, Arc length z = x = 3.0 μm, Applied voltage = 1 kV, Distance between the electrode = 0.5 cm and the electric field intensity E= V/d = 2 kV/cm, the induced Transmembrane Potential (TMP) is calculated as $\Delta\Phi i$ = 1.0779 volt. The results obtained from the analytical approaches are not more accurate because of the approximations done in the cell geometries and the shapes. So numerical method analysis is needed to get more accurate results to find the induced transmembrane potential. Transmembrane potential is defined as the potential difference between the inner and outer cell membrane which

$$\Delta\Phi_i = \Phi_{in} - \Phi_{out} \tag{1}$$

Where,

Φ_{in} = Potential at the inner membrane.

Φ_{out} = Potential at the outer membrane.

From the obtained results, the transmembrane potential which is the difference in the electric field intensity of the inner and outer membrane respectively and it was calculated as $\Delta\Phi_i$=0.9148V. The following Table 3 shows the calculation of induced transmembrane for the orientation angle of α = 90° using two different method namely analytical and numerical method.

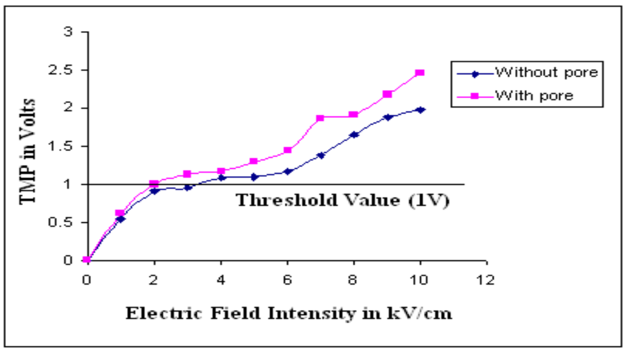


Figure 18. Variation of TMP under uniform field for ellipsoidal cell.

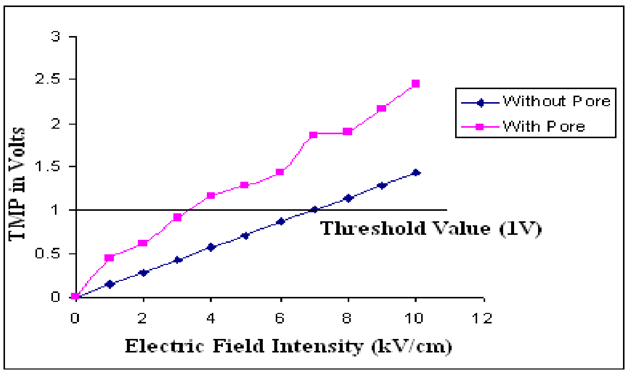
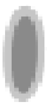


Figure 19. Variation of TMP for spherical cell under uniform field.

For a given electrode size and spacing between the electrodes, electrode shape as long as it is rounded or flat has no influence on electric field because of uniformity of the field. But the needled shaped electrode has influence on electric field due to non uniformity in the field which increases the inactivation rate. In this study, effects of electric field intensity for the bacterial cell with and without pore on its membrane for two electrode configuration was analyzed bacteria were analyzed using finite element method and the results are tabulated in Table 4 and 5. Figure 20 shows the variation of TMP under non uniform field for ellipsoidal and spherical cell.

Table 3. Induced Transmembrane potential for α = 90°

E orientation ↑	Analytical solution in volts	Numerical solution in volts	% error
α = 90°			
	1.0779	0.9148	15.13%

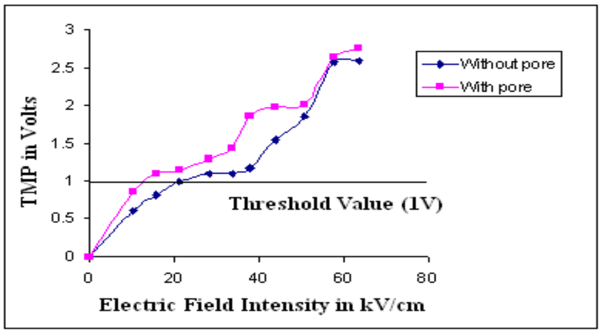


Figure 20. Variation of TMP under non uniform field for ellipsoidal cell.

Table 4. TMP for ellipsoidal Cell under non uniform field Utilization factor (η) = 0.127

Applied voltage In kV	Electric field intensity in kV/cm	TMP for Bacteria with out pore in volts	TMP for bacteria with pore in volts
0.5	10.276	0.6129	0.8554
1.0	15.742	0.8124	**1.0852**
1.5	21.276	**0.9935**	1.1363
2.0	28.250	1.0944	1.2870
2.5	33.848	1.1264	1.4372
3.0	37.931	1.1737	1.8542
3.5	44.064	1.5502	1.9793
4.0	50.853	1.8593	2.0082
4.5	57.979	2.5703	2.6243
5.0	63.764	2.6803	2.7531

Table 5. TMP for spherical cell under nonuniform Field Utilization factor (η) = 0.118

Applied voltage in kV	Electric field intensity (E) in kV/cm	TMP for Bacteria with out pore in volts	TMP for bacteria with pore in volts
0.5	20.765	0.3654	0.8554
1.0	25.623	0.4124	0.9043
1.5	31.208	0.6129	**1.0963**
2.0	39.832	0.9235	1.2870
2.5	45.653	**1.0944**	1.4372
3.0	56.741	1.1737	1.8542
3.5	61.664	1.5502	1.9793
4.0	67.521	1.8593	2.0823
4.5	69.456	2.1703	2.2243
5.0	74.328	2.4803	2.3531

6. Conclusion

The electric field analysis of bacteria (*E. coli*) with and without preexisting pore was carried out for uniform and non uniform field configuration and the induced transmembrane potential was calculated analytically and numerically. Based on the obtained results, the following conclusions were drawn.

- Under uniform field ellipsoidal cell with pore on its membrane requires an electric field intensity of 3 kV/cm whereas spherical cell with pore on its membrane requires 4 kV/cm which is proved from the finite element analysis.
- The reduced electric field strength of about 25% is required under uniform field configuration during the inactivation mechanism for the ellipsoidal cell with pre-existing pore on the membrane compared to spherical cell with pore on its membrane.
- As the breakdown of the cell membrane occurs at the induced transmembrane potential of 1V, the operating value can be achieved at the appreciably lesser voltage under uniform field for the ellipsoidal cell with pre-existing pore in the membrane compared to spherical cell.
- The numerical method provides 15% more accurate result when compared to the analytical method used for the calculation of TMP required for complete sterilization.
- Under non uniform field, the ellipsoidal cell without pore on its membrane needs the desired electric field strength of 15.72kV/cm to produce the transmembrane potential of 1V whereas the desired electric field strength is 4kV/cm under uniform field for the applied voltage of 1kV. Hence the inactivation rate increases with non-uniformity of the electric field and also depends on the electrode geometry.

7. References

1. Jayaram SH, Boggs SA. Optimization of Electroporation Waveforms for Cell Sterilization. IEEE Trans Ind Appl. 2004 Dec; 40(6):1489–97.
2. Gimsa J, Wachner D. Analytical Description of the Transmembrane Voltage Induced on Arbitrarily Oriented Ellipsoidal and Cylindrical Cells. Biophys J. 2001 Oct; 81(4):1888–96.
3. Qin BL, Canovasa GVB, Olsen RG. A Continuous Treatment Chamber System for Inactivating Microorganisms with Pulsed Electric Field. IEEE Transactions on Dielectrics and Electrical Insulation. 1995 Jan; 4(1).
4. Schulthesis C, Bluhm HJ, Mayer HG. Industrial Scale Electroporation of Plant Material Using High Repetition Rate Marx Generator. IEEE Transactions on Plasma science. 2002 Dec; 32(4).
5. Van Den Bosch HFM, Morchuis PHF, Smit JJ. Temperature Distribution in Fluids Treated by Pulsed Electric Field. Proceedings of 14th International Conference on Dielectric Liquids; Graz (Austria): 2002 July 7-12.
6. Schoenbach KH, Stark RH. Buescher ES. Bioelectric-New Applications for Pulsed Power Technology. IEEE Trans Plasma Sci. 2002 Feb; 30(1).
7. DeBruin KA, Krassowska W. Modeling Electroporation in a single cell. I. Effects of Field Strength and Rest Potential. Biophys J. 1999 Sep 22; 77(3):1213–24.
8. Sato M, Ishda NM, tri sugiarto A. High-Efficiency Sterilizer by High Voltage Pulse Using Concentrated Field Electrode System. IEEE Trans Ind Appl. 2000 Dec; 37(6):1646–50.
9. Pedrow PD, Olsen RG. Heat Conduction in Microbes Exposed to Pulsed Electric Field. IEEE Transactions on Dielectrics and Electrical Insulation. 1998 Dec; 5(6):878–85.
10. Bruhn RE, Pedrow PD, Olsen RG. Inactivating Microorganisms Using a Pulsed Electric Field Continuous Treatment System. IEEE Trans Ind Appl. 1998 Feb; 34(1):43–50.
11. Jayaram SH. Sterilization of Liquid Foods by Pulsed Electric Fields. IEEE Trans Electr Insul. 2000; 16(6):17–25.
12. Boggs SA, Zheng Z, Jayaram SH. Effects of Dielectric Saturation of Water on Transmembrane Potential in Biological cells. Biophys J. 2005 Mar; 21.
13. Wu T-F, Tseng S-Y, Hung J-C. Generation of Pulsed Electric Fields for Processing Microbes. IEEE Trans Plasma Sci. 2004 Aug; 32(4):1551–62.
14. U.S. Food and Drug administration. Kinetics of Microbial Inactivation for Alternative Food Processing Technologies, Pulsed Electric Field. Center for food safety and applied nutrition; 2002 Jun.
15. Creygton Y, Beurskens R, Fiala A. Power Source for Inactivation of Microorganisms with Partial High Voltage Discharge in a Continuous Process. IEEE Trans Plasma Sci. 2002.