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Electrical Field Modelling of *E. coli* Bacteria

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Abstract: In this study, an electrical model of *E. coli a* rod shaped bacterium causing diarrhea through food contamination, is developed using numerical methods to analyse the effects of electric field on it. The critical electric field intensity (Ec) of prolate cell model is calculated both analytically and numerically. The results show that the maximum Ec is obtained for cells oriented parallel to the applied field. The effect of product and microbial factors on Ec is also analysed. The results show that electro-permeabilization is not only a function of cell orientation, but also of product factors and microbial factors.

Key words: Electropermeabilization, prolate spheroid model, transmembrane potential, critical electric field intensity

INTRODUCTION

High voltage engineering finds its application in all branches of science, medicine and even in food technology. As an application of high voltage in food processing, high intensity short duration high voltage pulses are used for preserving the liquid food (Eynard *et al*., 1998). Exposing microbial cells to high voltage Pulsed Electric Field (PEF) may result in a dielectric breakdown of cell membrance. This breakdown can either be reversible or irreversible, depending on the intensity and duration of the applied electric field. Reversible breakdown has wide applications in science, biotechnology and medicine. Irreversible breakdown results in inactivation of micro-organisms like bacteria, yeast and viruses. Destruction of micro-organisms in liquids is of particular importance in the food industry, pharmaceutical research, public health and water purification (Eynard *et al*., 1998). Bacterial decontamination using intense electric pulses was first reported by Sale and Hamilton in 1967 and 1968 their conclusion are still valid.

Biological cells when placed in an electric field undergo local distortion of the field in the cell and in its vicinity. The electric field is mostly concentrated on the membrane. The membrane can be considered non conductive when compared to cytoplasm and physiological extra cellular medium having conductivities of several orders higher than the membrane (Kotnik and Miklavcic, 2000).

Analytical description of steady-state transmembrane voltage induced on spherical cells was derived more than four decades ago. To simplify the derivation Schwan assumed the membrane to be non conductive which leads to the well know relation often referred to as the (steady-state) Schwan's equation.

$$\Delta\phi = \frac{3}{2} ER\cos\theta \quad (1)$$

Where $\Delta\phi$ is the induced Trans membrane potential (TMP), E is the external field, R is the cell radius and θ is the polar angle measured from the center of the cell with respect to the field.

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Electroporation of the biological cells occur when the induced transmembrane potential (TMP) exceeds the critical electrical field (E_c).

The inactivation of microbes in liquid foods by PEF depends on many factors that are critical to the outcome of the process. The factors are basically classified as process parameters, product parameters and microbial characteristics. The recent reviews by Jayaram (2005) describe the effect of such parameters on inactivation, kinetics by pulse application. As given in the review, an in depth knowledge of these factors and their influence on the treatment is essential for improved PEF analysis and to avoid misinterpretation of results. The investigation of induced potential distribution on the cell membrane becomes vital in studying the electric field effect on biological cells.

Potential distribution on the surface of cells placed in an electric field can be calculated analytically or numerically. But the analytical methods give only a rough picture of the dependence of induced TMP on electric and geometric parameters (Teissie *et al*., 2003). Therefore it appears that only numerical methods give a sufficiently precise estimation of field values in realistic cell anatomies. But till date only very few studies of this type have been reported (Sebastian *et al*., 2001). The main reason is the difficulties faced in handling regions of very different size scales, microns for cell diameter and nanometers for the membrane thickness. The numerical solution to Laplace equation in the forms of finite differences involves a kind of polynomial approximation in nodes of a convenient grid, the existence of very small domains make it necessary to use a very dense grid or alternatively sophisticated non-uniform meshing methods. For this, power full Finite Element Method (FEM) has been used in this study. *E. coli* is a common bacterium that normally inhibits the intenstinal tracks of humans and animals. It is a gram negative, non-sporing rod shaped bacterium with 2 μm length and 0.8 μm diameter with 1 μm^3 volume size. The cell envelope of the cell is 20 um thick and has two membranes, an inner cytoplasm membrane and an outer lipopolyscacchride membrane (Laroussi *et al*., 2003).

In this study, the various parameters influencing the PEF treatment are simulated using a Field solver package. The emphasis is on the influence of these factors on the E_c necessary to cause a TMP resulting in a irreversible breakdown.

In this study, a prolate spheroid cell (2d) model (Wall *et al*., 1999) is developed using the FEM package and the critical electric field intensity is calculated numerically. The numerical results were validated with analytical calculations. Then the dependence of E_c on factors such as cell density, medium properties, electrode shape, size and spacing were analyzed using numerical methods.

Calculation of Critical Electric Field Intensity of Non-Spheroid cell

The threshold electric field, E_c is defined by Schwan's equation,

$$E_c = \frac{\Delta\phi_i}{1.5R\cos\theta} \tag{2}$$

where $\Delta\phi_i$ is the induced transmembrane potential, R the cell radius and θ the angle defined between the applied electric field and the point vector of the calculation on the cell membrane. Non-spherical cells such as rod shaped cells can be approximated as prolate spheroids. From the solution of the Laplace equation for the potential on an arbitrary oriented spheroid, the E_c values can be obtained for all orientations (Teissie *et al*., 2003). The E_c values for a non-conductive membrane can be written as a generalized Schwan equation,

$$E_c = \frac{\Delta\Phi_i}{\sin\alpha\dfrac{1}{1-L_x}x + \cos\alpha\dfrac{1}{1-L_z}z} \tag{3}$$

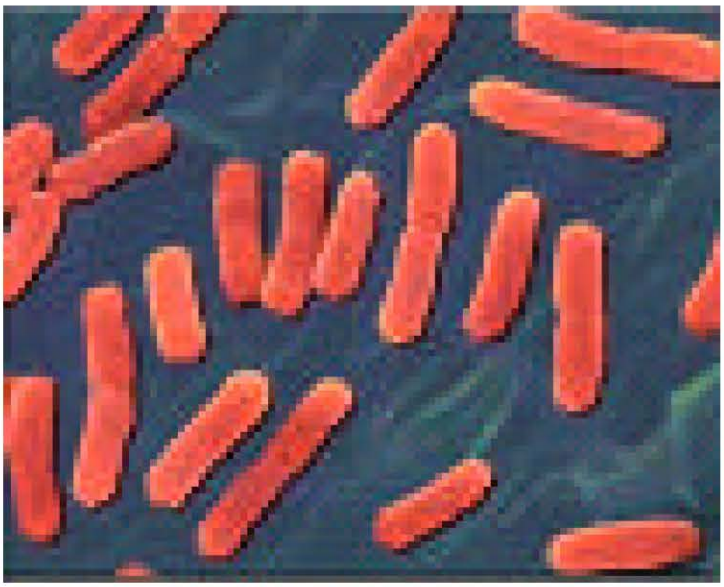

Fig. 1a: Photograph of an *E. coli*

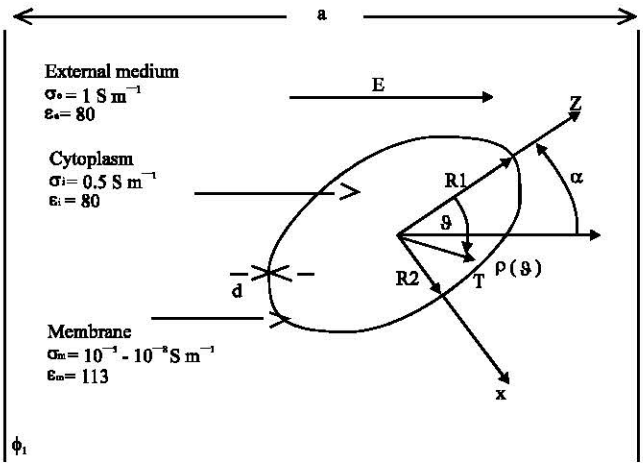

Fig. 1b: Schematic representation of *E. coli*

Where L_x and L_z are depolarizing factors, which depend only on the geometrical properties of the spheroid and $\Delta\phi_1$ is the induced transmembrane potential. The *E. coli* and its schematic representation are show in Fig. 1a and b where ϕ_1 and ϕ_2 are constant potentials, which are applied to induce a homogenous electric field. A cross section of a spheroid with the x z plane is shown (Fig. 1b). α is the angle between the electric field and the z axis of symmetry α and p(ν) is the arc length for a given angle ν. $R_1 = 2\ \mu m$, $R2 = 0.8\ \mu m$ and membrane thickness $d = 20$ nm.

The variables x and z are the coordinates of the point on the surface of the spheroid. The angle α between the symmetry axis of the spheroid and the external electric field defines the cell orientation with respect to the electric field.

MATERIALS AND METHODS

The objective of this analysis is to evaluate the electric field intensity required to induce the threshold transmembrane potential. The absolute value of the threshold transmembrane potential of *E. coli* is 1V (David, 1999). The methodology used in determining the E_c is discussed as below.

Cell Model

A simple prolate spheroid model of the *E.coli* was modeled using an FEM package. The cell consists of cell content or cytoplasm surrounded by a very thin non-conductive membrane and placed in a conductive medium. Since under normal conditions membrane conductivity is many orders smaller than that of external medium, it is neglected. This model and typical electrical properties of the materials are shown in Fig. 1a and b, respectively.

Finite- Element Analysis

A finite elements model of a cell in a medium is built for numerical analysis. For generation of models and calculations the program package MAXWELL (ANSOFT, Pittsburgh, PA) is used. FEM solves partial differential equations by dividing the volume into smaller elements and solving differential equation on elements. These elements have shapes and sizes so that complex geometries can be modeled (Teissie *et al*., 2003). Resolution of the method is increased by increasing number of elements. The maximum number of elements is limited by the computer memory capacity. All computations are performed on HP 7500.

To model an isolated cell, the spheroid is enclosed in a box, representing external medium, placed between copper electrodes with spacing 'a' as shown in Fig. 2.

The cell is exposed to a homogenous electric field by applying a constant voltage to electrodes as shown in Fig. 1b and natural boundary conditions are applied to the electrodes (ϕ_1=V, ϕ_2= 0 Volts). The transmembrane voltage $\Delta\phi$ induced by the external electric field on the cell membrane is the difference between the values of electric potential at the boundary surfaces.

$$\Delta\phi = \phi_i - \phi_e \tag{4}$$

where ϕ_i the potential induced on the interior of the membrane and ϕ_e is the potential induced on the exterior of the membrane.

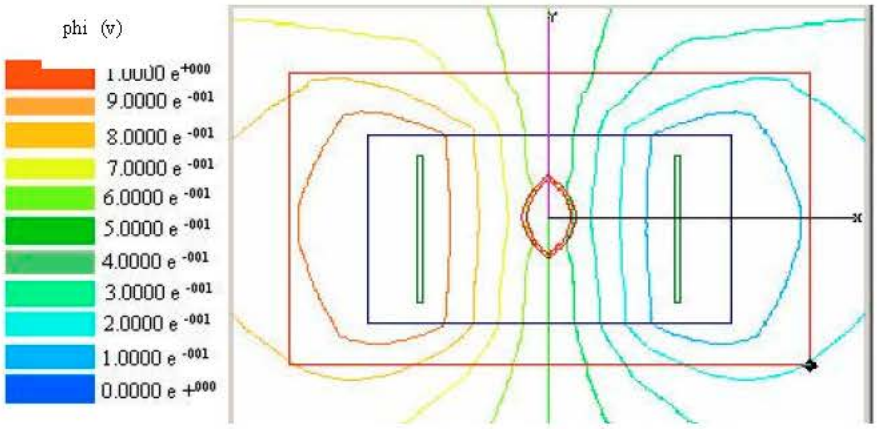

Fig. 2: Equipotential plot of prolate spheroid cell exposed to electric field

Table 1: Analytical and numerical solutions for the critical electrical field intensity

Angle of orientation α°		
Analytical solution E_c (kV cm^{-1})	2.75	1.35
Numerical solution E_c (kV cm^{-1})	3.10	1.65

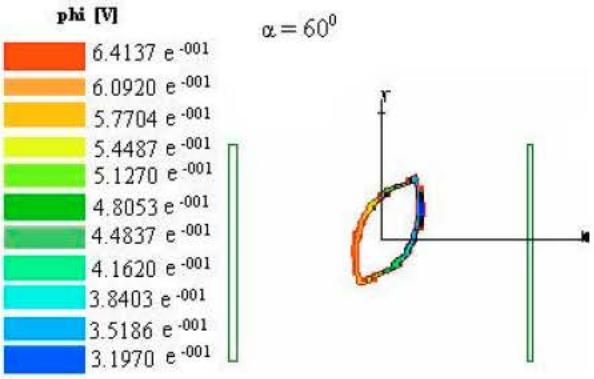

Fig. 3a: Transmembrane potential for orientation angle of 60 degrees

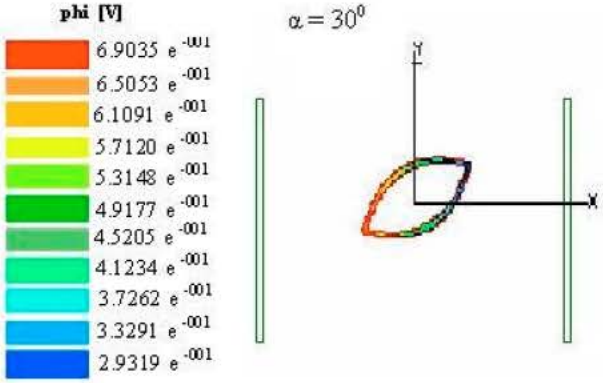

Fig. 3b: Effect of orientation angle on Trans- membrane potential for α = 30 degree

The equipotential flow through the medium that makes up the cell cytoplasm, the membrane and the electrolyte (culture medium) in which the cell is suspended is shown in Fig. 2. This shows that the highest potential difference across the membrane occurs at the cell ends along the major axis of the prolate spheroid which clearly supports the earlier studies stated in the literature.

The effect of the orientation angle on transmembrane potential is shown in Fig. 3.

For α = 0, α = 30, 60 and 90°. The critical electric field intensity of a prolate spheroid cell is calculated for different angles of orientation α between the electric field and the axis of symmetry, numerically, by using the finite elements method and analytically according to Eq. 2. The results as shown in Table 1 indicates that the FEM model shows similar potential distribution as that of realistic cell.

Since there is no analytic method showing the dependence of E_c on product factors, process factors and microbial factors (Wouters *et al*., 1999).

FEM model is used for this analysis and the results are discussed below.

RESULTS AND DISCUSSION

Effect of Process Factors on Critical Electric Field Intensity E_c

Effect of the Electrode Configurations

Electric field intensity is one of the main factors that influences electropermeabilization. The electropermeabilization increases with an increase in the electric field intensity, (Gowri Sree *et al*., 2005) above the critical transmembrane potential. The electric field intensity depends on electrode configurations (electrode size, electrode spacing) and electrode shape (Fig. 4). Different electro configurations were analysed and the results show that for a given electrode size, E_c is high for high spacing between the electrodes (Fig. 4).

Effect of Electrode Shape

From the results (Fig. 4) one can see that the electode shape is not significant on E_c. But for a given electrode size and spacing, the electric field uniformity varies with the shape of the electrode. Thus the angle of curvature was varied from minimum (Needle shape) to maximum (parallel plane). The needle shape electrode (1°C) has E_{max} 4.5 kV cm^{-1} in contrast to E_{min} 2.012 kV cm^{-1} (Fig. 5 for plane electrodes).

Effect of Product Factors

Electropermeabilization also depend on physico-chemical properties (medium conductivity, fat content) of suspending medium. The influence of the product factors on E_c is investigated one by one as detailed below.

Effect of Conductivity of Medium

The electrical conductivity of a medium (σ, S/m), which is defined as the ability to conduct electric current, is an important variable in electropermeabilization. Foods with large electrical conductivities generate smaller peak electric fields across the electrodes (Wouters *et al*., 1999).

An increase in the difference between the conductivity of a medium and microbial cytoplasm weakens the membrane structure due to an increased flow of ionic substance across the membrane. The Conduction solver solves for electric potential, Ø, in the continuity equation,

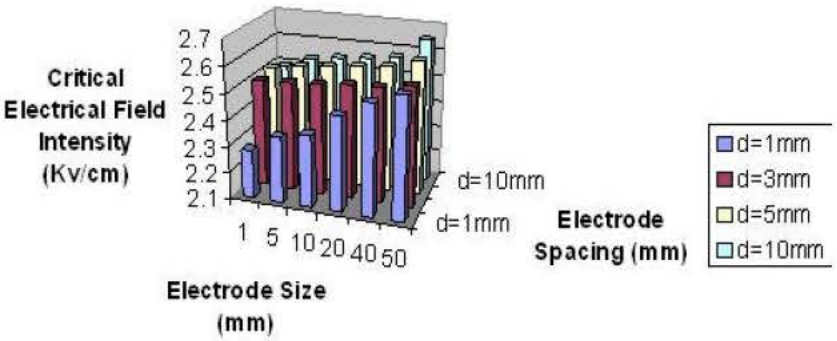

Fig. 4: Effect of process factor-electrode size and spacing

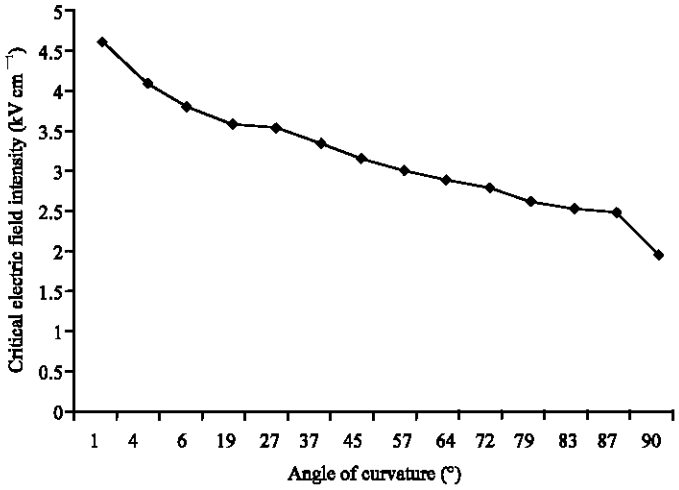

Fig. 5: Effect of process factors-electrode shape

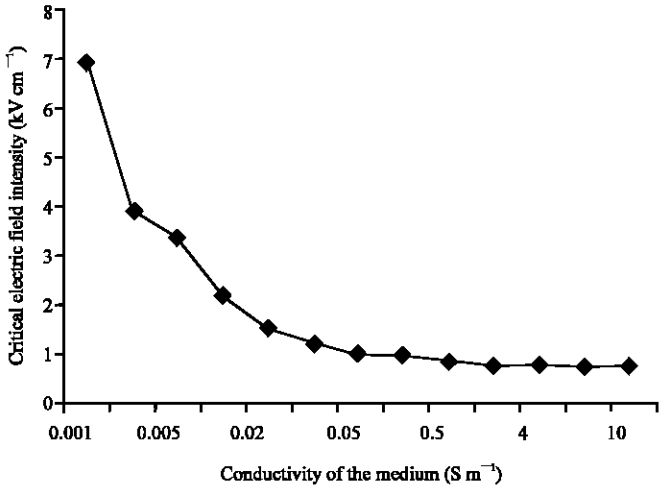

Fig. 6: Effect of medium conductivity on E_c

$$\nabla.(\sigma\nabla\phi) = 0 \tag{5}$$

The conductivity of medium (Luiro Broth) is increased from 0.001 to 10 S m^{-1}. From the Fig. 6, the medium with high conductivity requires lower critical electric field intensity E_c keeping other parameters as constant.

The nutrients (fat, protein) in medium (milk) also influence field distributions.

Effect of Medium Type

The electropermeabilization occurs when medium having different permittivity of that of cytoplasm is used, as the low permittivity material (membrane) is stressed more. Therefore the electropermeabilization of the *E. coli* model is analyzed with different mediums as described in Table 2.

Table 2: Critical electric field intensity of different mediums

Medium	Dielectric constant	Conductivity (S m^{-1})	Critical electric field intensity (kV cm^{-1})
Milk	69.5	0.50	3.15
Apple juice	72.5	0.18	2.90
Cherry juice	73.7	0.051	2.72
Fresh water	81.0	0.01	2.60
Orange juice	84.0	0.335	2.50
Grape juice	87.0	0.304	2.45

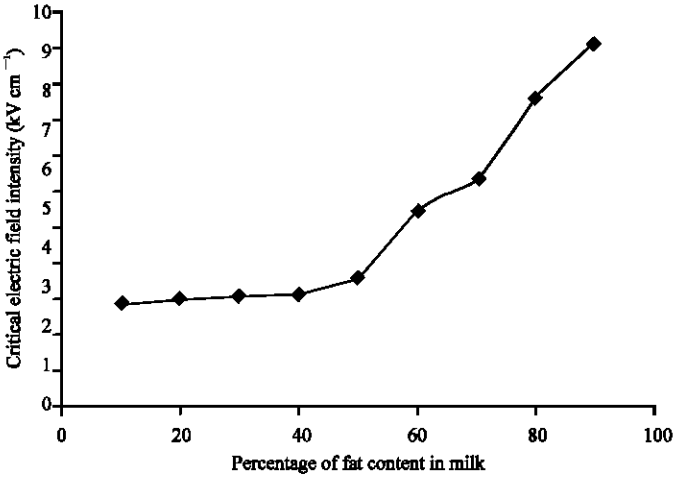

Fig. 7: Effect of fat content in milk on electric field intensity E_c

Effect of Fat Content in Milk

The fat content in milk also influence microbial inactivation. As the percentage of fat content in milk is increased the permittivity of the milk is increased. The permittivity of a mixture of two polar liquid dielectrics depends on the ratio of the components contained in the `mixture and it is calculated using "logarithmic law of mixing",

$$\log^{\epsilon}{}_{m} = y_1 \log^{\epsilon}{}_{1} + y_2 \log^{\epsilon}{}_{2} \quad (6)$$

Where

ϵ_m = Permitivity of the mixture
ϵ_1 = $\epsilon_o \epsilon_{r1}$
ϵ_2 = $\epsilon_0 \epsilon_{r2}$
ϵ_{r1} = Relative permittivity of the milk (69.5)
ϵ_{r2} = Relative permittivity of the soluable fat (3.1)
y_1, y_2 = Volume fraction of the constituents.

As the percentage of the fat increases, the resultant permittivity decreases and hence critical electric field intensity decreases. Therefore the percentage of fat content in milk is increased with constant conductivity and E_c is calculated. From the Fig. 7 the higher fat content in milk shows protective effect on *E. coli*. More fat, higher is the E_c required for the breakdown of the membrane. Hence the fat behaves as an insulator.

Effect of Water Content in the Milk

The above analysis is repeated with water ($\epsilon_r = 81$) having higher dielectric constant than milk. As the water content increases, the effective permittivity increases and hence the E_c decreases (Fig. 8). For an increase of 80% in water content, the E_c decreases by 13.5%.

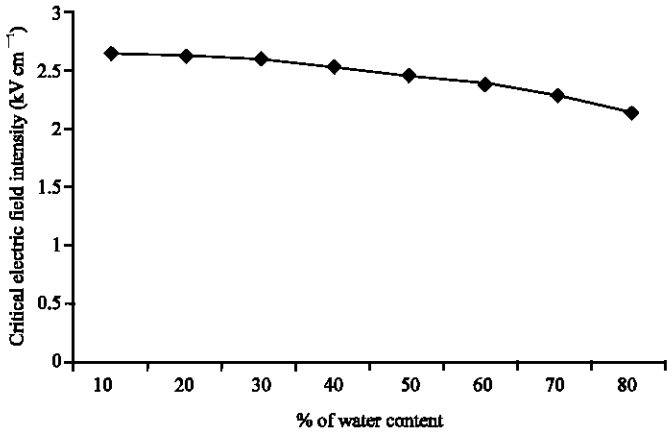

Fig. 8: Effect of water content in milk on electric field intensity E_c

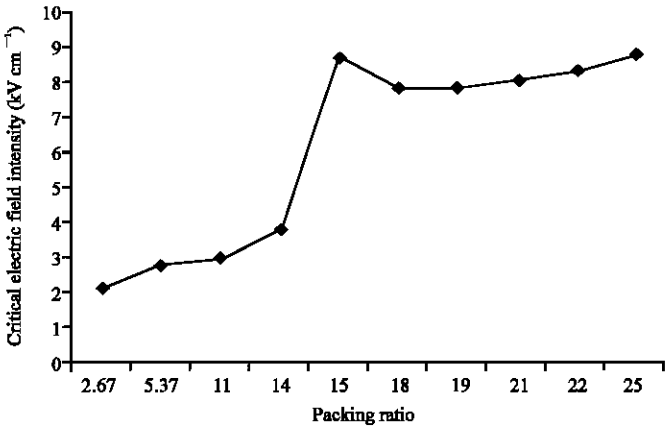

Fig. 9: Effect of packing ratio on critical electric field intensity E_c

Effect of Microbial Factors on E_c

Effect of Cell Density

Since other cells always surround the cells, mutual interactions between the cells influence the critical electric field intensity. The cell density is expressed in terms of Packing Ratio (PR). The Packing Ratio is defined as a ratio of cell distance to cell diameter. For rod shaped cells, the longest radius (R_1) is taken as equivalent cell radius (Pavlin *et al*., 2003). Higher value of PR represents dilute cell suspensions.

From the Fig. 9, the diluted cell suspensions require higher electric field intensity for effective permeabilization than the denser cells.

CONCLUSIONS

The electrical model developed is validated, by comparing with analytical results. The effective permeabilization requires high medium conductivity, low fat content and also a needle shaped electrodes with smaller spacing influence this process. Also the inactivation rate increases with cell density and with non-uniformity of applied field. Therefore the dielectric modeling constitutes an important tool for the field analysis of biological cells.

Using these results we can improve the experimental process and validate the experimental datas more accurately as the model is available for all the effecting parameters influencing the PEF analysis. This will definitely improve the analysis process of microbial inactivation by PEF treatments with better understanding of the physics of the problem.

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