DIELECTROPHORETIC DEVICES FOR SEPARATING FOODBORNE PATHOGENS

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Abstract. *Today an increased interest in bioparticle separation research field is shown. Microtechnology and nanotechnology opens new perspectives in food quality analysis such as bioMEMS (Micro-Electro-Mechanical-Systems) devices for simultaneous detection of microorganism in food products with improved sensitivity and velocity [1].*
Diseases caused by different foodborne pathogens such as bacteria, viruses, fungi, parasites, etc. have been a serious problem.
Classical microbiological methods are taking a long time to confirm results for a particular pathogen organism. At present numerous rapid methods are being studied, for example polymerase chain reaction (PCR) [2], enzyme linked immunosorbent assay (ELISA) [3], dielectrophoresis (DEP) [4,5], dielectrophoresis combined with ELISA, dielectrophoresis combined with electro rotation (ROT), travelling wave dielectrophoresis, etc.
Dielectrophoresis is a method of manipulation of a micro particle in an electric field gradient which results from the interfacial polarization [6]. Using low voltages and temperatures below 39 degrees allows us no permanent damage to the cells.
Theoretical modelling of behaviour in electric field is very important for the applications we need. Electrode arrangement from bioMEMS and channel geometries affects abilities to separate foodborne pathogens.
This article describes the results obtained by our research team for manipulating bacteria with a specific electrode type of DEP.

Keywords: *bioparticle, dielectrophoresis, separation*

Introduction

The ability to accurately control and handle micrometer and nanometer scale particles is intensively studied in recent years especially as a capture method in different fields, including the food industry. [7]
One of the methods used for this purpose is dielectrophoresis - DEP. Dielectrophoresis movement consists in a bioparticle capture caused by an electric field applied.

Dielectrophoretic force depends on the electrical properties, geometrical and morphological factors and environmental bioparticle of suspension and the applied electric field characteristics (frequency and intensity).
It may be highlighted both in continuous current (DC) [8] and in alternating current (AC) [9-14] because dielectrophoretic force does not depend on the electric field polarity [9]. In literature the most commonly used is the alternating electric field [10-14].

Dielectrophoresis as electrokinetic phenomenon that utilizes an asymmetric electric field to sort different bioparticle like nano-pathogen agents was performed using a micro fabricated device [11]. Separation was possible through operation of the characteristically properties differences between normal and infected cells. Sorting experiments were placed inside a microchip made from different microelectrodes patterned on a glass substrate.

In this study we establish the experimental arrangement for capture lactic bacteria with dielectrophoretic forces. This method is not destructive, which is a great advantage.

Theory

The basic law which describes the forces acting on charged particles in electric field:

$$\vec{F} = q\vec{E}(\vec{r} + \delta\vec{r}) - q\vec{E}(\vec{r})$$

where q = Q_+ = Q_- is the dipole electric charge and $\delta\vec{r} = d$ is the distance between charges.

The behavior of a dipole in a variable electric field is described in figure 1 and the movement of particles under the effect of the resultant force in a uniform phase (a,b) and variable phase (c) is described in figure 2.

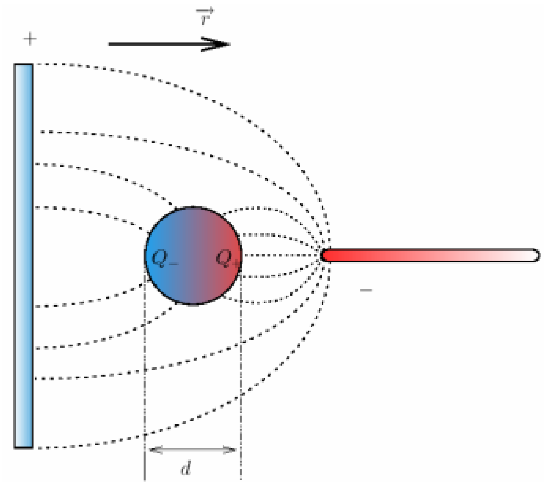

Fig.1 Dipole placed in irregular electric field [16]

In the nonlinear medium as biological medium, the dependence between electric field *E* and polarization *P* has the following form:

$$P = \alpha E + \alpha_1 E^2 + \alpha_2 E^3 + \ldots ,$$

Size called molecule polarizability, is a physical quantity numerically equal to the induced dipole moment in the molecule by an electric field intensity unit.

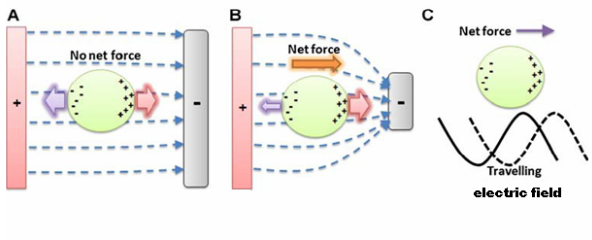

Fig. 2 (a) Uniform electric field resultant force acting on the particle is zero.
(b) A particle is under a DEP force because of gradient electric field intensity.
(c) A particle is under a DEP force because of a gradient electric field phase [14]

Cases:

a. If α does not depend on the direction of electric field orientation, the molecule is isotropic in terms of electric polarization.
   Polarization vector is in this case:
   $$\vec{P} = N\vec{p} = N\alpha\vec{E} = Nq\vec{l} ,$$
   N is the number of molecule, q is charge molecule and l is the distance between positive center charge distribution and negative center charge distribution;
b. If α polarizability molecule varies with electric field orientation, the molecule behaves an anisotropic induced dipole moment and the electric field inducing a relationship tensor form:
   $$p_i = \alpha_{ik} E_k \ \ cu\ (i,k = 1,2,3),$$
   α_{ik} is the components polarizability tensor molecule;
c. In anisotropic media with different properties after different directions:

$P_k=\varepsilon_0\chi_{ki}E_i$, cu $(i,k=1,2,3=x,y,z)$,

is the electric susceptibility tensor components of the environment:

$$\chi_{ik}=\begin{pmatrix}\chi_{xx} & \chi_{xy} & \chi_{xz}\\ \chi_{yx} & \chi_{yy} & \chi_{yz}\\ \chi_{zx} & \chi_{zy} & \chi_{zz}\end{pmatrix}=\begin{pmatrix}\chi_{11} & \chi_{12} & \chi_{13}\\ \chi_{21} & \chi_{22} & \chi_{23}\\ \chi_{31} & \chi_{23} & \chi_{33}\end{pmatrix}$$

For charged particles and electric field polarized in the absence of magnetic field, the force has two components, electrostatic force and dielectrophoretic force.

$$\overrightarrow{F_{tot}}=\overrightarrow{F_{es}}+\overrightarrow{F_{def}}=q\vec{E}+(\vec{P}\cdot\nabla)\vec{E}$$

If there is a gradient electric field, then it will cause a net shift of the charged particles. The dielectrophoretic force must overcome Brownian motion.

This relationship establishes a requirement on the minimum particle size that can be manipulated, so high values of gradient electric field are necessary to manipulate the order of nano-size particles.

Next we express dielectrophoretic force (DEP) in a three dimensional configuration. In an AC electric field, E(t) is a harmonic function of time and the force can be write as:

$$F=\Gamma\varepsilon_m\left[\mathrm{Re}(f_{cm})\nabla(E_{xo}^2+E_{yo}^2+E_{zo}^2)+\mathrm{Im}(f_{cm})(E_{xo}^2\nabla\varphi_x+E_{yo}^2\nabla\varphi_y+E_{zo}^2\nabla\varphi_z)\right]$$

where Γ is the geometric factor of the particles, ε_m is the dielectric constant of suspending medium, and f_{cm} is the Clausius–Mossotti factor. The terms $\mathrm{Re}(f_{cm})$ and $\mathrm{Im}(f_{cm})$ refer to the real and imaginary parts of f_{cm} [8].

The DEP force has two major terms: the first term represents the “classical DEP force” the second term represents the “travelling-wave DEP force”.

For a spherical particle the $\mathrm{Re}[f_{cm}]$ is determined by taking the real component of the complex form of the Clausius–Mossotti factor:

$$f_{CM-sferic}=\frac{\varepsilon_p^*-\varepsilon_m^*}{\varepsilon_p^*+2\varepsilon_m^*}=\left(\frac{\sigma_p-\sigma_m}{\sigma_p+2\sigma_m}\right)\left[\frac{j\omega\frac{\varepsilon_p-\varepsilon_m}{\sigma_p-\sigma_m}+1}{j\omega\frac{\varepsilon_p+2\varepsilon_m}{\sigma_p+2\sigma_m}+1}\right]$$

where ε_p^* and ε_m^* are the conjugate complex dielectric permitivities of the particle and medium, respectively and σ_p is the conductivity of the particle, σ_m is the conductivity of the medium and ω is the angular frequency of the applied electric field [15].

Results and discussion

We aimed to study the behaviour of lactic acid bacteria, particularly lactobacillus and streptococcus species.

For this purpose we used a starter culture Yoghurt (YO-MIX 495 LYO 100 DCU, Danisco, Sassenage, France)[12,13,16].

After the standard procedure, the suspension of bacteria is put under the microscope. We follow the behaviour of bacteria under the influence of electric field.

The DEP force allows particles to move independently of their charge in an inhomogeneous electric field applied to the micro device (chamber electrode). Materials constituting the dielectrophoretic chamber electrode are complex (for example: Pt Pd Au Mo Cr Al). It is very difficult to choose the material which the electrodes are made from. Electrode geometry, their number and distance between them are important parameters in choosing the experimental set-up.

To avoid the complexity of the manufacturing process, only one material is usually used to manufacture electrodes. Nowadays different combinations of materials are used. The selection of materials for manufacture of electrodes depends on the desired destination, ionic species involved, environmental impact of materials and their suitability to manufacture

In our study we established to generate a variable electrical field and to serve as DEP electrodes for capturing bacterial cells to the electrode surface.

We performed an experimental set-up composed of alternating signal source, a dielectrophoretic chamber made as a result of collaboration between UMF Bucharest, “Vasile Alecsandri" University of Bacau and MGM STAR CONSTRUCT SRL. Several electrodes images are shown in figure 3(a, b, c).

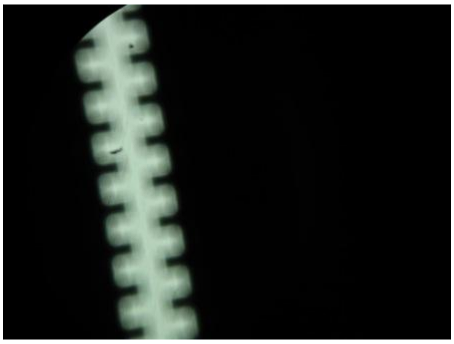

Fig. 3a Dielectrophoretic chamber with “teeth” electrodes. This image is made at biophysics department UMF Bucharest

Fig. 3b Dielectrophoretic chamber with linear electrodes. This image is made at biophysics department UMF Bucharest

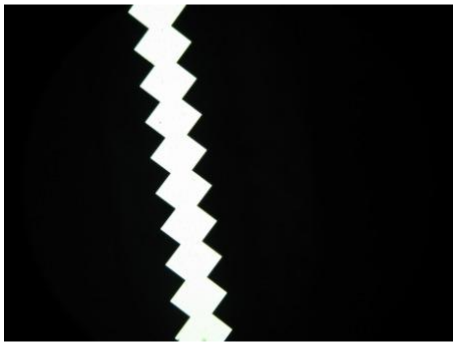

Fig. 3c Dielectrophoretic chamber with castellated electrodes. This image is made at biophysics department UMF Bucharest

In this study the electrodes of dielectrophoretic chamber were made from chromium, with a thickness of about one quarter micron. It is very difficult to produce technologically the space between electrodes, which must provide electrical isolation on the one hand, and on the other hand must have a good regular dielectrophoretic effect. We made different types of electrodes. Images are viewed with optical microscopy at UMF Bucharest and AFM microscope at “Vasile Alecsandri" University of Bacau.

AFM applications in cell biology can be classified into several broad categories: imaging as shown in figure 4, micromanipulation studies, material property measurements and binding force measurements. In this study we are using AFM to view the lactic bacteria behaviour.

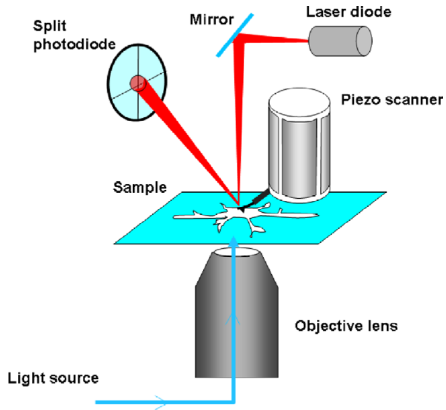

Fig.4 Scheme of an AFM coupled with an inverted optical microscope [17]

Imaging of living cells has been coupled with controlled culture systems, enabling the possibility of continuous, long-term imaging.

An important feature of AFM is the possibility of studying dynamic processes at high spatial resolution [17].

The AFM images of a micro channel electrode which has handled the bacteria are presented in figure 5 a, b:

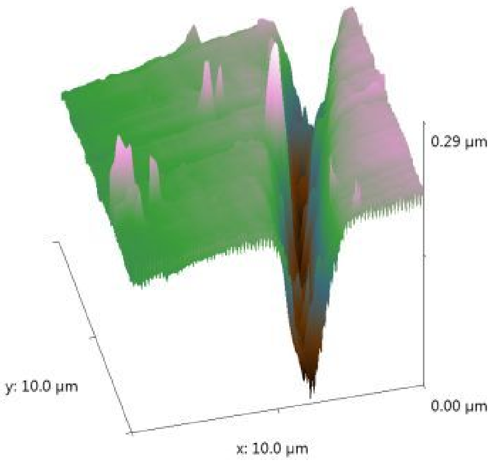

Fig. 5a The image of a micro channel with 0.29 µm thickness

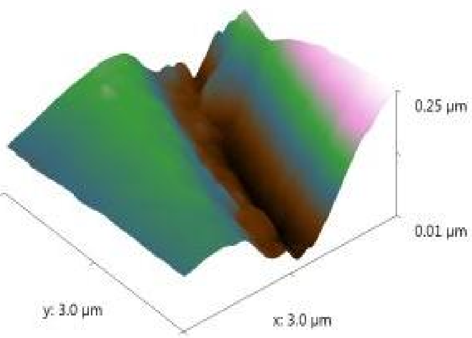

Fig. 5b The image of a micro channel with 0.25 µm thickness

Figure 6 shows the experimental set-up accomplished for capture lactic bacteria with DEP force and figure 7, the dielectrophoretic chamber.

Fig.6a Experimental set-up

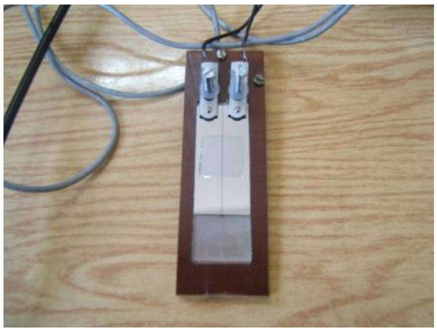

Fig.6b Dielectrophoretic chamber

Conclusions

The first steps in a DEP experimental set-up were established for lactic acid bacteria, particularly lactobacillus and streptococcus species.

We determined the optimal geometric configuration for the electrodes between which a maximum electric field gradient is applied.

We made an experimental arrangement which allows the variation of frequency and voltage applied to study the influence of this pulse of electric current on bacteria.

A number of investigations by optical microscopy lactic bacteria migration were observed and the first results are encouraging, but working with so many variable parameters is a difficult task.

In the future we intend to establish the experimental conditions in which lactic bacteria capture is achieved by positive dielectrophoresis (DEP); the method allows concentration of lactic bacteria in a sample.

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