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FACTORS AFFECTING DIELECTROPHORETIC SEPARATION OF CELLS USING HIGH-GRADIENT ELECTRIC FIELD STRENGTH SYSTEM

Z. ZAINAL ABIDIN*[1], D. AWANG BAIK[1], N. HAFIFUDIN[1], G. MARKX[2]

[1]Department of Chemical and Environmental Engineering, Faculty of Engineering, UPM, 43400 Serdang, Selangor, MALAYSIA
[2]School of Chemical and Analytical Science, University of Manchester, Sackville Street, M60 1QD, Manchester, UK
*Corresponding Author: zurina@eng.upm.edu.my

Abstract

An investigation on dielectrophoretic separation of cells has been conducted using high-gradient electric field system (HGES). The HGES system consisted of two concentric cylindrical electrodes whereby the space between them was filled with glass beads. The glass beads were found to distort the electric field generated between the two electrodes and thus creating a high field gradient sites that produce dielectrophoretic force for cells collection. In order to study the effectiveness of the system in separating the cells, a series of experiments have been conducted. Here, yeast cells were introduced into the system and the number of cells collected was measured. The effects of voltage, flow rate, type of matrix, height of matrix and sample concentration have been investigated. In addition, the electric field analysis for the HGES has also been carried out using FEMLAB. Results show that the cells collection is influenced by the effect at the condition with and without electric field. Further analysis on the investigating factors enabled one to predict optimum values for voltage, flow rate, type of matrix, and height of matrix and sample concentration in order to improve the efficiency of the system by reducing the effect when no field is applied.

Keywords: Dielectrophoresis, Cell Separation, Electric Field, High Gradient.

Nomenclatures

$V_{pk\ -\ pk}$	Peak-to-peak voltage
∇E^2	Gradient of the square of the electric field strength
DEP	Dielectrophoresis
HGES	High-gradient electric field system

Greek Symbols

ε_p	Particle permittivity
ε_m	Medium permittivity
ε	Electrical permittivity

1. Introduction

Improvement in the separation of microbes has always been a necessity in order to accommodate the advances made in bio- related discipline. The use of dielectrophoresis (DEP) as a basis to separate particles based on their dielectrical properties has shown results with great potential. Dielectrophoresis works by inducing the motion of matter suspended in a fluid in a non-uniform electric field [5]. It can have negative effect when the particle permittivity, ε_p is greater than medium permittivity, ε_m ($\varepsilon_p > \varepsilon_m$) or positive effect when the particle permittivity, ε_p is smaller than medium permittivity, ε_m ($\varepsilon_p < \varepsilon_m$).

High gradient electric field strength technique (HGES) introduced by Lin and Benguigui [2,3,4] utilizes the DEP principles. In this technique, matrices are placed between the energized electrodes, resulting in regions of highly non-uniform electric field being form around the matrices. Fluid containing particle flowing inside this system will experience a net DEP force. This method has been shown to be useful for filtration [1] and separation [2, 3, 4, 6, 7]. In this work, we will investigate the characteristics of the HGES system on several parameters in order to establish optimum conditions for HGES to work efficiently.

2. Materials and Methods

2.1 Preparation of cells

Saccharomyces cerevisiae (strain 239, isolated from Whitbread the brewers, obtained from Mr. Ralph Cooper, University of Manchester) was grown overnight in 100 ml MYGP broth containing 0.3 % each of yeast extract and malt extract (Oxoid), 1 % of glucose and 0.5 % of mycological peptone (Oxoid), at 35°C in an orbital shaker with a speed of 150 rpm. The cells were centrifuged (Int. Equipment, model Centra 4MPR) and washed four times and resuspended in deionised water to reduce the conductivity. The conductivity was checked using a Jenway conductivity meter (model 4010). The concentration of the cells was measured using a UV spectrophotometer (PYE Unicam 8600, Philips) in a cuvette of 1 cm path length before doing the experiments.

2.2. Preparation of HGES chamber

The HGES chamber is constructed using two co-axial cylindrical electrodes made of stainless steel. The inner electrode has an inner diameter of 1mm and the outer electrode has an inner diameter of 4.7 mm. The HGES chamber or column has a length of 50 mm. Co-axial connectors were used as covers at the top and bottom in order to seal the column. The top co-axial connector has a small rod in the middle for attaching and centering the inner electrode (Fig. 1). A syringe needle which served as the inlet port for inflow was attached at the top by piercing it through the plug. A nylon filter (Millipore, UK)of 30 μm pore size and had a hole on it for outlet flow was placed at the bottom plug in order to support the matrix bed in the column. Glass beads and Barium titanate were used as matrices. Glass beads were obtained from Sigmund Lindler GmbH, Germany and had a size distribution of in the range of 40-70 μm. Barium titanate comes from Alfa Aesar, Johnson Matthey, Germany and had a size distribution of 23 μm to 63.5 μm.

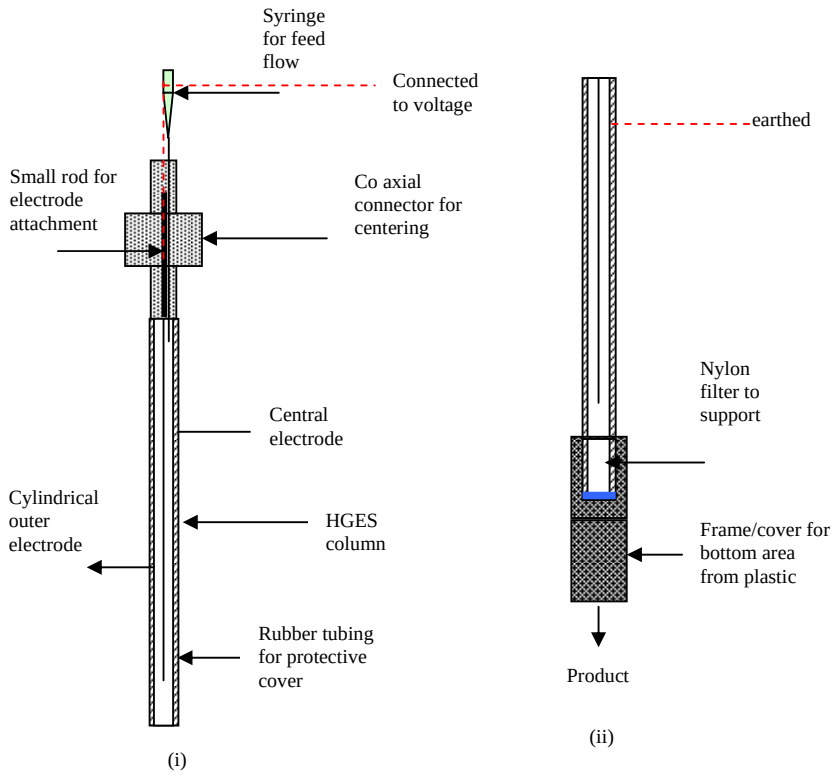

Fig.1. High Gradient Electric Field Strength Column for DEP Separation of Sells. Top section is shown in part (i) and bottom section in (ii). The outer electrode is tapered at both open ends.

2.3 Experimental procedures

In this study, effects of voltage, flow rate, heights of matrices and type of matrices were investigated. Initially, the electric field (0 v – 60 v and 1 mhz) was applied prior to the start of the experiment using a standard frequency generator (Thurlby-Thandar, TG120) with a self-built high frequency amplifier. 1 ml of yeast suspension with concentration of 0.108 (optical density value) was injected into the HGES column and the yeast cells were attracted to the high field regions by positive DEP. Next, a deionized water flow of between 120 $\mu lmin^{-1}$ to 300 $\mu lmin^{-1}$ was introduced into the chamber using a Sage Syringe Pump (Model 355, USA) to wash away the cells that were not captured. Heights of matrices were varied from 0 to roughly 28 mm. Finally, the outlet suspension was collected for 30 minutes and the cell concentration was then measured. Experiments were also repeated to test the effect of using glass beads (electrical permittivity, $\varepsilon = 4.5$) and barium titanate ($\varepsilon = 1600$).

2.4 Electric field calculations

The electric field calculation of the electric field strength, E was done at dc conditions using FEMLAB software version 2.3 (Comsol Ltd). The simulations were conducted for voltage 30 $V_{pk\text{-}pk}$ and 1 MHz with the inner and outer electrode diameter being 1.0 mm and 4.7 mm.

3. Results and Discussion

The investigation was started by studying the effect of voltage on the dielectrophoretic separation of yeast using a chamber with an inner electrode having a diameter of 2 mm and an outer electrode having a diameter of 4.7 mm. The voltage was varied while keeping the frequency at 1 MHz. Results in Fig. 2 illustrates the behaviour of the percentage of the total yield with respect to the change in voltages. Without the application of the electric field, the number of cells trapped around 48 % (2.3×10^6 cells/ml). This calculated mechanical yield actually contributed quite a considerable amount to the total yield. Almost half of the cells inputs were collected without the influence of the dielectrophoretic force. When no electric field was applied, our high gradient electric field column was similar to a deep bed filtration system. Normally, the particles to be trapped are smaller than the size of the packing. At 0 V, some of the cells were trapped between the interstices of the matrix due to inertia. The mechanical yield will therefore very much dependent on the way the bed of packing being arranged. In this case, the sizes distribution of the glass beads used was 40-70 μm and they were packed randomly to give a porosity of 0.44**.** Nevertheless, the occurrence of the mechanical trapping reduced the porosity of the bed. In other words, this means that less space is available between the pores of the glass beads to capture cells when the electric field is applied. Figure 2 also indicates that upon the application of 20 V, the percentage total yield increased to 56 % is achieved. Although the increment of the yield is relatively small, this is enough to show that the DEP-HGES concept worked in the column designed. Further increment of the electric field resulted to an increase of the total yield to 80 % at 60 V.

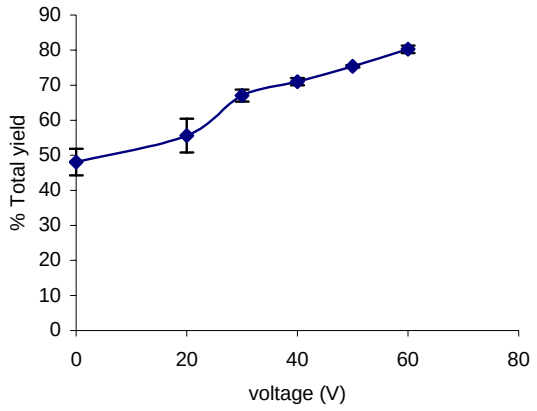

Fig.2. Changes of the Percentage Total Yield in regards versus Voltage.

The mechanical yield calculated at 0 V is 48 % which make up quite a significant amount to the initial cells injected.

The glass bead has a dielectric permittivity value of 4.5. When the electric field is applied, a non-uniform electric field is created between the electrodes due to their configurations and geometry. The presence of the glass beads between the electrodes, distorts the electric field pattern further (Fig. 3). The non-uniformity is created in the neighbourhood of the glass beads elements and vanishes in the distances comparable to the diameter. The maximum electric field occurs at the point of contact of beads. The cells are collected this location and also at the beads surfaces. This prevents the matrix from bridging across the electrodes and shorting out the electric field. As the time of operation increases, the accumulation of the particles on the bead surfaces due to the DEP, the effect of the fluid flow rate and the pressure drop across the bed will reduce the porosity. This in turn will also reduce the separation efficiency of the HGES-DEP column as all the free spaces available has been filled up by the cells. Analysis on the ∇E^2 values as in Fig. 4 shows the electric field strength to decline exponentially with the distance from the centre of electrode and its magnitude increases with the increase in voltage.

Figure 5 displays the variation of the total yield with respect to flow rates with and without electric field application. At both 0V and 30V, the total yield decreased with increased in the flow rates. With no electric field applied, the cells to be captured are entrapped in the interstices among glass beads held in a deep bed as mechanical yield. The application of electric field has increased the amount of cells collected. There are many competing forces acting upon cells such as gravitational, drag and also DEP forces which results in net force acting on the cells and determine the movement of the cells. If the drag force is less significant than the DEP force which holds the cells at the matrix, then the cells would remain intact at the beads surfaces. However if the drag force is greater, then the cells will be washed from the column. Increasing flow rates increases the drag force that washed the cells from the HGES column.

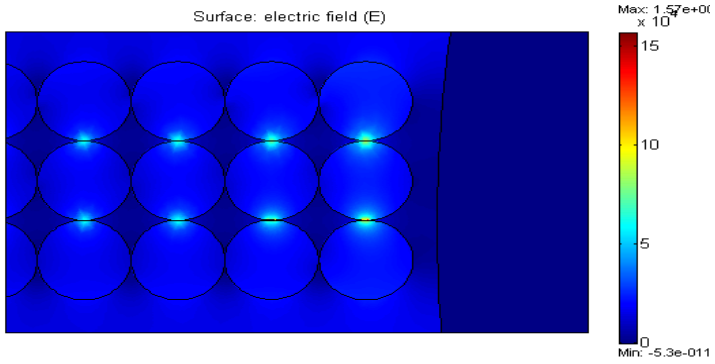

Fig. 3. Electric Field Distribution inside the HGES Column when the System is Filled with Beads.
High electric field regions are indicated by red and yellow. The high field regions can be found at the points of contact of the beads.

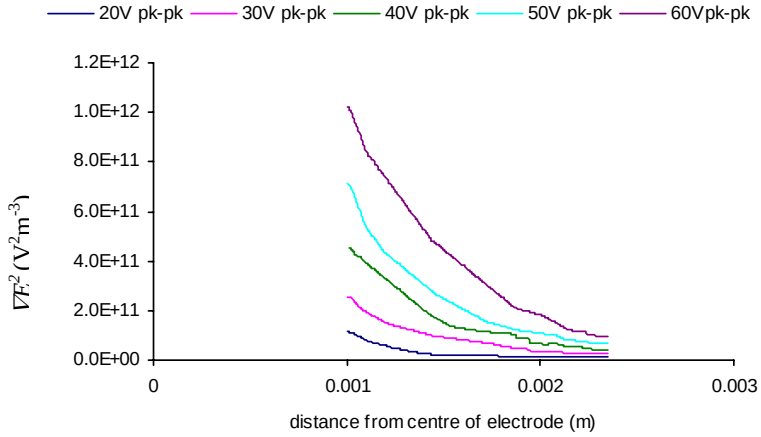

Fig.4. ∇E^2 as a Function of the Distance from the Centre of the Electrodes Calculated using FEMLAB. The value of ∇E^2 appears to decline exponentially with the distance from the centre of electrode. Also, as expected, its magnitude increases with voltage.

Figure 6 meanwhile shows the changes of the percentage total yield with respect to voltage for two different types of matrices that are barium titanate and glass beads. At 0V, the mechanical yield of the system for barium titanate is higher compared to the other two matrices simply because the particle size distribution for the barium titanate is smaller (23 μm - 63.5 μm) compared to glass beads (40 μm - 70 μm). This generates smaller pore size between interstices

of barium titanate bed compared to glass beads which in turns affect the mechanical yield. When electric field was applied, the total yield increases proportionately and increased with the voltage. Figure 7 shows the electrical yield variation for the two matrix. Electrical yield is defined as the total yield minus the mechanical trapping. Barium titanate gave the highest electrical yield of 27 % in comparison to glass beads at 60 V. It is believed that this is due to high permittivity value of barium titanate which indicates its high polarizability upon the application of non-uniform electric field. This effect causes the electric field to be distorted and thus produce a high-gradient region compared to glass beads which helps to enhance the collection of the cells. The electric field is believed to be more concentrated on barium titanate powder compared to the glass beads which have a lower value of polarizability.

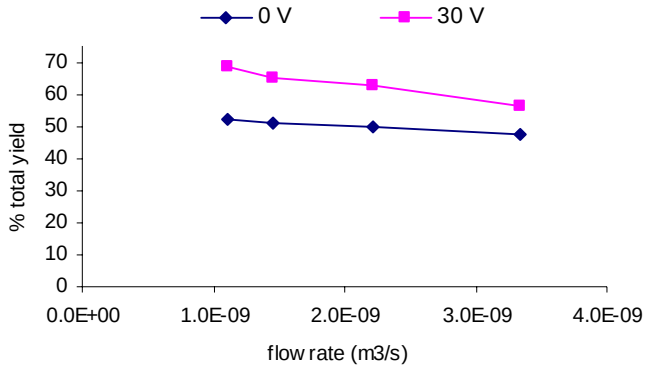

Fig. 5. The Variation of the Total Yield versus the Change in the Flow Rate with and without Electric Field. The total yield decreases as the flow rates increases both at 0V and 30V.

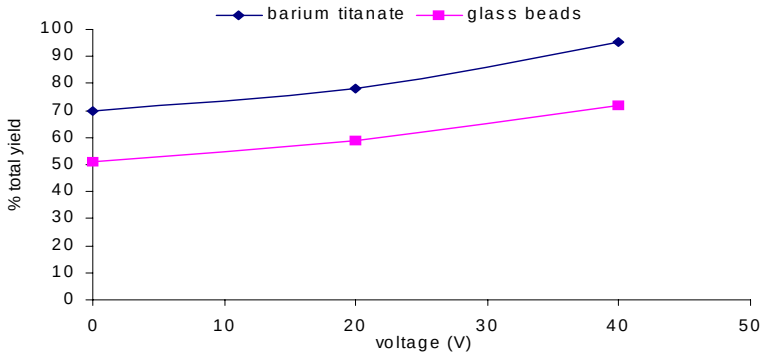

Fig. 6. Changes of the Percentage Total Yield versus voltage for Two Different Types of Matrices, Barium Titanate and Glass Beads.

The mechanical yield of the system for barium titanate is higher compared to the other two matrices.

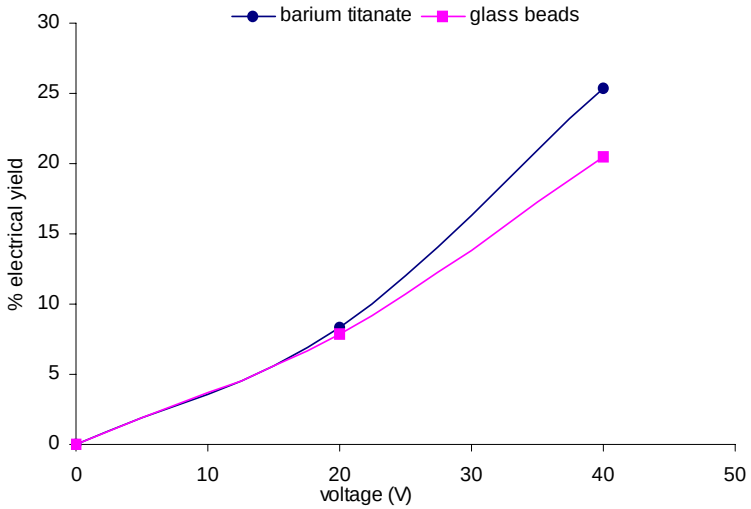

Fig. 7. Electrical Yield Variation versus Voltage for Three Different Types of Matrices.

The highest % electrical yield was obtained for barium titanate compared to stainless steel and glass beads packing.

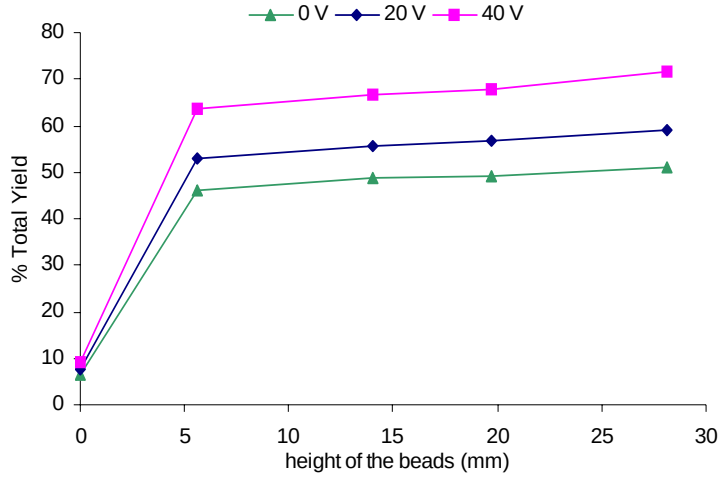

Fig. 8. Percentage Total Yield versus Heights of the Beads.

The mechanical yield at 0 V increased with respects to increase in the heights of the packed bed. The same patterns are reflected when the electric field is applied and also when the magnitude of the electric field is increased.

When the height of the beads is increased, both the total yield at 0 V and 30V also increases (Fig. 8). The same patterns are reflected when the magnitude of the electric field is increased. The increment in the bed height will increase give more cell mechanical as well as electrical trapping. This is because more glass beads means more site available for the cells to be captured when being electrified. Furthermore, more glass beads also means more point in contacts between them for cells collection. On the other hand, too many beads will eventually retard the flow of the fluid towards the end of the chamber and reduce the efficiency of the HGES column.

4. Conclusions

The results of the HGES system have been presented and discussed here. Basically, a DEP chamber made from two concentric cylindrical columns had been constructed. The inner cylinder was connected to voltage supply while the outer electrode was grounded. The empty space between the two cylinders was filled with glass beads. The beads play the role of distorting the electric field and generate a high gradient site inside the column for cells trapping as shown by the electric field analysis done using FEMLAB. A few parameters have been selected to study the feasibility of the DEP separation system. It was found that the mechanical trapping at zero voltage attributed to deep bed filtration process made up to a significant amount of 40% of the total yield. This is obviously not good but it is still believed this technique is still viable to be further developed and improved. As the voltages increased, the electrical yield also increased. A sufficiently high electric field gradient site has been generated around the beads for cell attachments. Results indicated that ∇E^2 as high as 10^{15} V^2m^{-3} was recorded at the point of contact of the beads. Meanwhile the ∇E^2 at the vicinities of the beads were also high of around 10^{10} – 10^{14} V^2m^{-3}. These values were extremely encouraging since they are of the same range of values found in the microelectrodes system. The cell collection distribution is supposedly to be proportional to the electric field strength patterns in the column. Therefore, more cells should be captured by bead with higher electric field strength resided near the central electrode than bead located further away. However, this prediction may not be entirely true since there is mechanical yield influence. The faster the flow rates resulted in a greater drag force and hence lesser electrical yield. As more beads resided inside the column, the mechanical and the electrical yield also becomes greater. Too many beads will eventually retard the flow of the fluid towards the end of the chamber. The larger the permittivity value of a matrix bed helps to enhance the electrical yield as shown by barium titanate compared to glass beads. The difference in the electrical permittivities for both barium titanate and glass beads also induced different electric field distributions around the beads and affects the electrical attachments of cells. In conclusion most of the results found using the FEMLAB modelling compliment the experimental findings and this helps us further in visualizing and explaining the behaviour of the HGES-DEP column system. Although the HGES column performance depends on most of the parameters investigated, however, the optimum characteristics and conditions for any DEP separation process is determined by nature of the feed to be separated. The HGES column can easily be built or modified for a greater capacity which runs at a more selective optimized condition for any particular

separation system. All in all, the HGES column has provide us with a different alternative to DEP separation of cells.

Acknowledgement

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