Paweł KIEŁBASA[1], Anna MIERNIK[1], Tomasz DRÓŻDŻ[1]

Faculty of Production and Power Engineering, University of Agriculture in Krakow (1)
ORCID.

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Effect of constant electric field stimulation of suspensions of selected microorganisms on geometric structure of cells

Abstract. *This paper discusses the effect of constant electric field on the geometrical parameters of selected microorganisms. In the experiment different time of exposure of suspension of microorganisms to constant electric field was applied and then the cell area and its diameter were determined. It was found that with increasing stimulation time the diameter and area of the cells relatively decreased in the case of Enterococcus faecalis strain ATCC 29212 as well as Candida krusei strain ATCC 14243. It should be noted, however, that in the case of Enterococcus faecalis ATCC 29212 cells the area and diameter of the cells after stimulation are higher than in the case of untreated cells, while in the case of Candida krusei ATCC 14243 both the area and diameter of the cells after stimulation are lower than in the cells from the control sample.*

Streszczenie. *W artykule omówiono wpływ oddziaływania stałego pola elektrycznego na parametry geometryczne wybranych mikroorganizmów. W doświadczeniu zastosowano zróżnicowany czas ekspozycji zawiesiny mikroorganizmów na oddziaływanie stałego pola elektrycznego a następnie określono powierzchnię komórki oraz jej średnicę. Stwierdzono, że wraz ze wzrostem czasu stymulacji średnica i pole powierzchni komórek względnie zmniejsza się zarówno w przypadku komórek szczepu Enterococcus faecalis ATCC 29212 jak również szczepu drożdżaka Candida krusei ATCC 14243. Należy jednak zaznaczyć, że w przypadku komórek Enterococcus faecalis ATCC 29212 pole powierzchni i średnica komórek po stymulacji jest wyższa niż u komórek nie poddanych takiemu oddziaływaniu, natomiast w przypadku szczepu Candida krusei ATCC 14243 zarówno pole powierzchni jak i średnica komórek po stymulacji jest niższa niż u komórek z próby kontrolnej (**Wpływ stymulacji stałym polem elektrycznym zawiesin wybranych mikroorganizmów na strukturę geometryczną komórek**)*

Keywords: electric field, microorganisms, cell size, production engineering
Słowa kluczowe: pole elektryczne, mikroorganizmy, wielkość komórek, inżynieria produkcji

Introduction

Electromagnetic fields have a wide range of applications in various branches of life - in medicine, agriculture and the food industry and the use of electric and magnetic fields can have both positive and negative effects on the functioning of living organisms [1-3]. Electric fields are used successfully in the food industry for pasteurization. This method was first used to pasteurize milk. The duration of the discharge is the same as that of an atmospheric discharge, and its intensity is 200 kV/cm. The electric field can be applied directly and indirectly. Direct application is where food products are exposed to the field, resulting in the inactivation of microorganisms by irreversible electroporation. When an electric field is applied to a cell, a change in the trans membrane potential is induced at the cell membrane, which can cause biochemical and physiological changes in the cell. When the threshold value of the trans membrane potential is exceeded, the cell membrane becomes permeable [4]. The spatial distribution of voltage across the cell membrane of biological cells has a number of theoretical and experimental implications, such as activation of voltage-gated membrane channels, cardiac cell stimulation, and cell membrane electroporation [5,6]. The efficiency of the process depends on the intensity of the electric field, the number of pulses and their duration. After using this method, food products do not lose their taste qualities [7].

Electroporation is a process associated with the induction of an extra transmembrane potential [8]. Electroporation can be a reversible or non-reversible process. Several factors directly affect the efficiency of electroporation. These factors can be divided into three main groups - factors dependent on the treatment conditions (electric field strength, treatment time and treatment temperature), factors dependent on the characteristics of the microbial units (type of microorganisms and their concentration) and media dependent factors (pH and conductivity of the medium) [4]. Irreversible electroporation of the cell membrane causes the efflux of soluble intracellular compounds of different molecular weights and ultimately leads to cell death [9-13]. Electroporation of isolated cells and dilute suspensions in vitro is affected by both the parameters of the electrical pulse(s). As the pulse amplitude increases further, the percentage of pored cells increases while the percentage of cells surviving treatment decreases. The average number of molecules introduced into the cell peaks at a certain average pulse amplitude. It has also been shown that both portion and and cell survival as functions of pulse amplitude vary significantly between different cell types. Some of the observed differences can be attributed to differences in cell size, but several studies suggest that differences in membrane composition and structure may also play an important role [8].

Cell death can be defined as the irreversible loss of integrity of the membrane [14]. Based on morphological criteria, three types of cell death have been distinguished in mammalian cells. Type I apoptosis, which is manifested by characteristic changes in the morphology of the nucleus, including chromatin condensation and fragmentation, changes in cytoplasmic organelles, and general shrinkage of the cell, rupture of the plasma membrane, and formation of apoptotic bodies containing nuclear or cytoplasmic material, leading to loss of plasma membrane integrity and subsequent cell death. Type II is characterized by massive accumulation of double-membrane autophagic vacuoles in the cytoplasm [15]. While type III known as necrosis, is a passive and pathological process. This type of death can occur under the influence of both physical, chemical and biological factors, including low and high temperature, UV radiation, and bacterial toxins. For cell death by necrosis to occur, the duration and intensity of the harmful agents must exceed the immune threshold of these cells. Necrosis affects cells that swell and lose cell membrane continuity [16]. In cells that undergo necrotic death, ATP levels decrease dramatically as a result of mitochondrial membrane depolarization, resulting in impaired electron transport. ATP deficiency disturbs the normal course of many processes [17]. The necrotic process also leads to destruction of organelles, including endoplasmic reticulum, polysomes, cell nucleus and lysosomes. The disruption of the cell membrane structure results in passive influx of water and ions into the cell [18-19]. The swollen cell and its organelles disintegrate, and all contents are released into

the intercellular space [20]. The structure of such material can be observed using for example the optical coherent tomography as in [21,22]

The aim of this study was to determine the effect of constant electric field on geometric parameters of selected microorganisms. In the experiment, different time of exposure of microorganisms to constant electric field was applied and the measured parameters were cell area and cell diameter. Knowledge of the relationship between the impact of a constant electric field on the cell will allow for precise adjustment of the exposure time, which may lead to the formation of pathological features in the cells and consequently their death.

Material and methods

The strains used in this study were from the strain collection of the Laboratory of Experimental Research Techniques of Raw Materials and Biological Products: *Candida krusei* ATCC 14243 and *Enterococcus faecalis* ATCC 29212 which are representatives of spherical microorganisms. In order to restore vital functions, the tested microorganisms were reductively inoculated on TSA solid medium. The procedure was carried out using a laminar flow cabinet creating sterile conditions to eliminate unwanted contamination. The cultures were maintained for 24 hours at 37°C. Suspensions of the test microorganisms were then prepared with an optical density of 0.6 on the McFarland scale.

The optical density of the sample was measured using a DEN-18 densitometer. A high-voltage pulse generator was used to conduct tests with the application of a pulsed electric field, allowing its smooth adjustment in the range from 0 to kV. The applied control system allowed for intermittent operation of the device with adjustable time interval of interruption as well as the number of generated pulses (Fig. 1).

Fig. 1: Constant electric field exposure stand

The interaction with a constant electric field took place in a chamber with two flat electrodes. The space between the electrodes allows stimulation of biological material placed in a Petri dish made of dielectric material. This arrangement allowed the material to be stimulated with a field of 3 kV/cm. After placing the dishes between the electrodes, the material was subjected to an electric field. The samples were subjected to an electric field with an intensity of 3 kV/cm in three combinations of time of exposure of the material to the aforementioned field, i.e. one, two and three hours in a continuous system. After the end of the stimulation of the samples with the constant electric field, intravital slides of the studied microorganisms were prepared and viewed under an OPTIKA B-510 microscope with PROVIEW x64 software, 3.7.13483.20181206. Using PROVIEW software, the size of the cells represented by their diameter and surface area was measured (Figure 2). The number of cells measured was 30 for each sample of the biological substance tested.

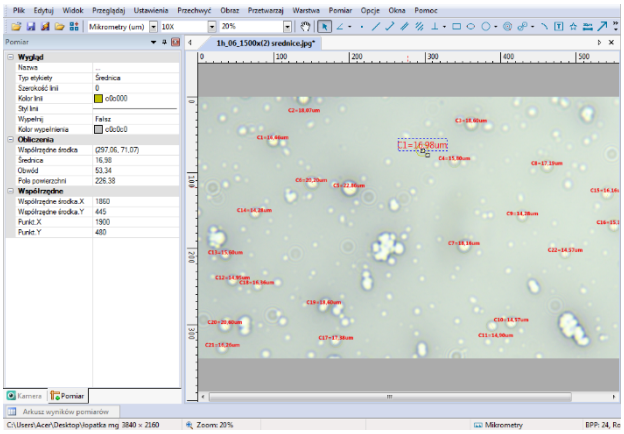

Fig. 2. PROVIEW Software

Results

In the case of spherical cells, the identification of shape parameter variations allows for a quick assessment of the effect of the stimulus, which was the application of a constant electric field. It was found that after stimulation of cells of the yeast *Candida krusei* ATCC 14243 with a constant electric field of 3 kV/cm there was a decrease in the diameter of the cells of this microorganism (Fig. 3).

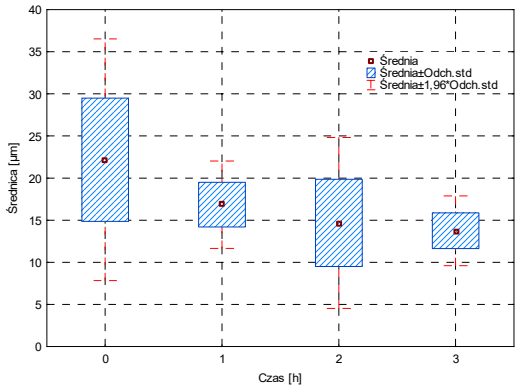

Fig. 3. Diameter of *Candida krusei* ATCC 14243 cells after exposure to a constant electric field of 3 kV/cm

It should be noted that with the increase of constant electric field stimulation, the diameter of *Candida krusei* ATCC 14243 cells decreased in comparison with the diameter of cells in the reference group, i.e. not exposed to the above mentioned electric field. After an hour of stimulation a decrease in cell diameter by 5.3 µm was obtained. On the other hand, the lowest cell diameter value was observed after a three-hour stimulation, which was 13.8 µm. In this case, the diameter decreased by 8.4 µm. Using Scheffe's test, statistical analysis of the differences in mean values was performed at a significance level of 0.05, which indicates, statistically significant differences occurred only between the diameter of cells from the control group and the other groups of cells that were given exposure to the constant electric field. No differences in cell diameter were observed between the groups with different times of constant electric field stimulation. It should also be noted the significantly smaller range of variation in the diameters of cells stimulated with a constant electric field compared to the diameters of cells not stimulated.

The opposite relation to that described above was observed in the case of constant electric field stimulation of *Enterococcus faecalis* ATCC 29212 cells, where the field intensity was also 3 kV/cm, because it was observed that after the application of a constant electric field the diameter

of the examined cells increased in comparison with the diameter of cells not subjected to such an action. It is worth noting that, similarly to *Candida krusei* ATCC 14243, as the stimulation time increases, the diameter of the test cells decreases proportionally to this time (Figure 4). After one hour of stimulation, the highest diameter value of 12.1 µm was obtained, which is 4.1 µm higher than that of the control sample. The lowest increase in diameter was obtained after a three-hour stimulation, where the diameter increased by only 1.4 µm.

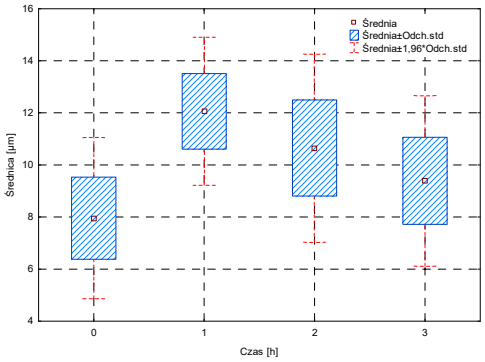

Fig. 4. Diameter of *Enterococcus faecalis* ATCC 29212 cells after exposure to a constant electric field with an intensity of 3 kV/cm

As with *Candida krusei* ATCC 14243 cells, the Scheffe test was also used to identify statistically significant differences in the mean values of *Enterococcus faecalis* ATCC 29212 cells. Significant statistical differences in mean cell diameter values were found between all combinations of constant electric field treatments and the null group. The variation of *Enterococcus faecalis* ATCC 29212 cell diameters by the action of a constant electric field is possible and statistically identifiable, allowing detailed studies to be conducted to generate characteristics to implement this technology in industry.

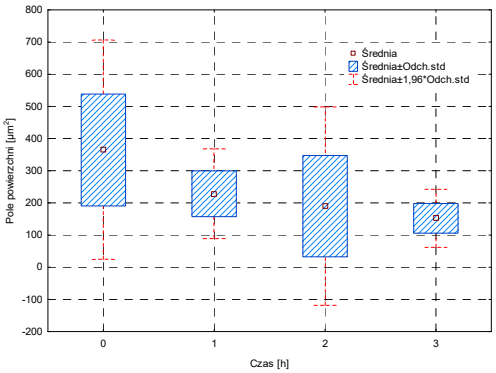

Fig. 5. Surface area of *Candida krusei* ATCC 14243 cells after exposure to a constant electric field with an intensity of 3 kV/cm

Another geometric parameter measured was the cell surface area calculated as the projection of the cell outline on the surface. It was found that after stimulation of cells of the yeast *Candida krusei* ATCC 14243 with a constant electric field of 3 kV/cm there was a significant reduction in the cell surface area of this microorganism in comparison with the unstimulated sample. Similar to the diameter, as the stimulation time increased, the surface area of the test cells decreased compared to that of the unstimulated cells (Figure 5). After a one-hour stimulation, there was a 1.5-fold reduction in cell surface area compared to the control sample and it was 228.3 µm2. In contrast, the lowest cell surface area of 152 µm2 was observed after three hours of exposure to a constant electric field.

For the cell surface area of *Candida krusei* ATCC 14243, statistical differences were found only between the constant electric field stimulated samples and the control sample, while there were no statistically significant differences within the constant electric field stimulated samples. Thus, the effect of stimulation on cell area size can be concluded, but the duration of exposure of the constant electric field to the microorganisms is not statistically confirmed.

On the other hand, analyzing the behavior of geometrical parameters of *Enterococcus faecalis* ATCC 29212 cells with a constant electric field of 3 kV/cm, an increase in the cell surface area of this microorganism was observed regardless of the duration of exposure (Fig. 6).

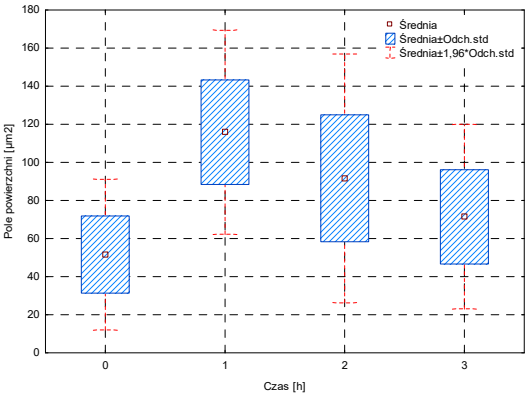

Fig. 6. *Enterococcus faecalis* ATCC 29212 cell surface area after exposure to a constant electric field with an intensity of 3 kV/cm

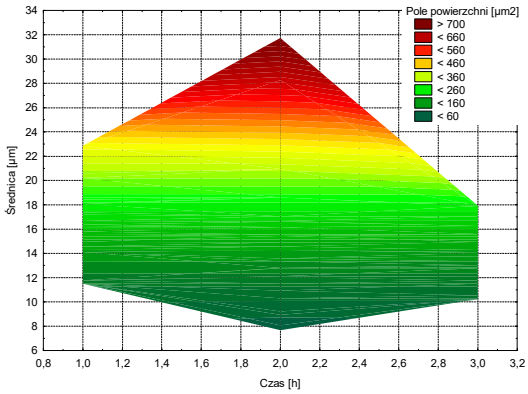

Fig. 7. Cell surface area of *Candida krusei* ATCC 14243 cells considering time interval of constant electric field and cell diameter

Thus, after the application of a constant electric field, the surface area of the test cells increased compared to the surface area of the cells not subjected to this interaction. It should be noted, however, that as in the case of *Candida krusei* ATCC 14243 cells, the surface area of the tested cells decreased with increasing stimulation time. After one hour of stimulation, a twofold increase in the surface area of 115.8 µm2 was obtained compared to the control sample. The lowest surface area was obtained after three hours of stimulation, where the cell surface area was 71.4 µm2. Analysis of *Enterococcus faecalis* ATCC 29212 cell surface area differences showed statistically significant differences in between all combinations of constant electric field interaction and the null group, which allows us to consider constant electric field interaction as prospective in stimulating *Enterococcus faecalis* ATCC 29212 cell surface area size.

Figures 7 and 8 visualize the relationships between the time of application of the constant electric field to the microbial suspension, cell diameter, and cell surface area.

In the case of *Candida krusei* ATCC 14243 cells (Figure 7), it is clear that a suspension stimulation time of two hours generates the highest surface area with a relatively large cel diameter.

Similar characteristics were observed in cells from *Enterococcus faecalis* strain ATCC 29212, (Figure 8) where it is also clear that a suspension stimulation time of two hours generates the highest surface area with a relatively large cell diameter, but it should be noted in this case that the differences recorded were statistically significant.

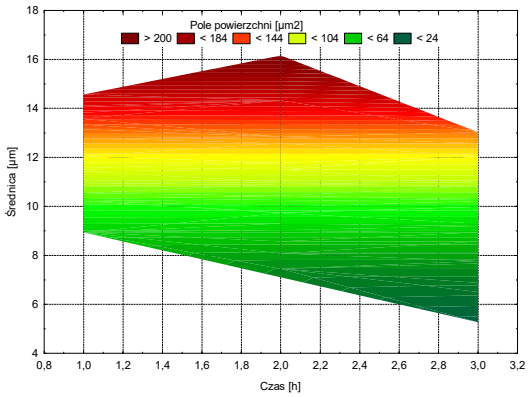

Fig. 8. *Enterococcus faecalis* ATCC 29212 cell surface area considering time interval of constant electric field and cell diameter

Conclusion

The diameter and area of the cells were found to decrease with increasing stimulation time, with Enterococcus faecalis ATCC 29212 having a higher area and mean of the cells after stimulation than the unstimulated cells. In yeast strain Candida krusei ATCC 14243 cell death is caused by electroporation - in the cell wall under the influence of an electric field pores are formed through which intracellular substances escape from the cell, As a consequence, cell shrinkage occurs. In case of Enterococcus faecalis ATCC 29212 cells cell death occurs by necrosis. Under the influence of electric field stimulation of cells, damage to important cell organelles occurs - swelling of mitochondria, resulting in inhibition of active ion transport across the membrane, which leads to the formation of perforations and absorption of water and mineral salts by the cells, resulting in the cell swelling increasing its size.

***Authors**: Paweł Kiełbasa Assistant Profesor, University of Agriculture in Krakow, Faculty of Production and Power Engineering,Balicka Av. 116B, 30-149 Krakow, E-mail: pawel.kielbasa@urk.edu.pl; Anna Miernik MSc Eng, University of Agriculture in Krakow, Faculty of Production and Power Engineering, Balicka Av. 116B, 30-149 Krakow, E-mail: anna.miernik@urk.edu.pl; Tomasz Dróżdż Assistant Profesor, University of Agriculture in Krakow, Faculty of Production and Power Engineering, Balicka Av. 116B, 30-149 Krakow, E-mail: tomasz.drozdz@urk.edu.pl*

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