Irreversible electroporation of bacterial cells L5

Duša Hodžić, Maša Kandušer
University of Ljubljana, Faculty of Electrical Engineering

Duration of the experiments: 60 min, 30 min the following day for results
Max. number of participants: 4
Location: Microbiological laboratory
Level: Basic

PREREQUISITES

Participants should be familiar with Safety rules and Rules for aseptic work in microbiological laboratory. No other specific knowledge is required for this laboratory practice.

THEORETICAL BACKGROUND

Irreversible electroporation is lately being tested as potential alternative treatment of different water samples. Short, high voltage electric pulses cause cell membrane disruption and lead to loss of viability and reduced number of bacteria in the treated sample. Different water samples were treated by irreversible electroporation and recently the method was tested also for hospital wastewater. Nevertheless optimization of electric pulse parameters is crucial for effective treatment.

Irreversible electroporation is a phenomenon based on the fact that when the cell is exposed to the external electric field of sufficient amplitude and duration, its membrane is permeabilized. Increasing amplitude of electric field increases the level of cell membrane permeabilization. When electric field parameters used are below the critical value, after some time cell membrane reseals and treated cells survive. If electric pulses exceed the critical value, the damage cannot be repaired and as a result cell viability is affected. The process is known as irreversible electroporation and is also used in alternative food processing technologies.

The efficiency of irreversible electroporation can be monitored by plating the treated sample on nutrient agar and counting the number of colony-forming units (CFU). Each colony may arise from a group of cells rather than from one individual cell and they represent the cells that survived electric pulse treatment.

The aim of this practical exercise is the demonstration of irreversible electroporation obtained on the sample of Escherichia coli resuspended in distilled water and exposed to different electric pulse parameters.

EXPERIMENT

We will detect irreversible electroporation by CFU count. The effect of electroporation on reduced cell viability will be determined for chosen sets of electric pulse parameters.
Our experimental organism will be E.coli K12 ER1821, cultured 12-16 hours in LB broth at 37 °C with vigorous shaking.

On the day of experiment 20 ml of bacterial cell culture will be centrifuged at 4200 rpm and the pellet will be resuspended in 20 ml of distilled water. Negative control will be the untreated suspension of bacteria. To determine the number of bacterial cells you will need to prepare serial dilutions of resuspended bacteria ranging from 10^{-1} to 10^{-8}. Dilute 100 µl of

bacterial suspension in tubes containing 900 µl sterile distilled water. First plate the negative control. Pipette 100 µl of dilutions 10^{-6}, 10^{-7}, 10^{-8} on LB agar in Petri dishes and spread them evenly with sterile glass rod.

For electroporation of the samples use dilution 10^{-1}. Electric pulses will be applied with electric pulse generator HVP-VG (Igea, Italy). Samples to be treated are placed in cuvettes with integrated aluminium electrodes, electrode distance 1 mm. The volume of the treated sample is 100 µl. After electroporation take 50 µl of the sample from cuvette for serial dilutions. Spread 100 µl of dilutions 10^{-4}, 10^{-5}, 10^{-6} on LB agar in Petri dishes and spread it evenly with sterile glass rod. All agar plates will be incubated overnight at 37 °C.

Count colony-forming units 24 hours after the treatment. From the data obtained determine the reduction of bacterial survival. When calculating the CFU/ml take into consideration all dilutions that were made, so the number of bacteria in original water sample can be estimated.

Number of viable cells per ml = number of colonies × dilution factor

Negative control represents total cell count and you will calculate the reduction of cell survival for samples exposed to different electric pulse parameters.

FURTHER READING:

Teissie J. et al. Recent biotechnological developments of electropulsation. *Biolectrochemistry* 55: 107-112, 2002.
Gusbeth C. et al. Pulsed electric field treatment for bacteria reduction and its impact on hospital wastewater. *Chemosphere* 75:228-33, 2009.
Saulis G. Electroporation of cell membranes: The fundamental effects of pulsed electric fields in food processing. *Food Engineering Reviews* 2:52-73, 2010.

NOTES & RESULTS

electric pulse parameters (E [V/cm], t (µs, ms), n, frequency [Hz])			
number of viable cells per ml			
reduction of E.coli [order of magnitude]			