E. coli inactivation in a continous flow system L7

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Duration of the experiment: day 1: 90 min; day 2: 60 min
Max. number of participants: 4
Location: Microbiological laboratory
Level: Basic

PREREQUISITES

Participants should be familiar with the Safety rules and Rules for sterile work in cell culture laboratory. No other specific knowledge is required for this laboratory practice.

THEORETICAL BACKGROUND

The first description of the profound effect of electrical pulses on the viability of a biological cell was given in 1958. If a cell is exposed to a sufficiently high electric field, its membrane becomes permanently permeable, resulting in leakage of cellular components, which leads to cell death. The method gained ground as a tool for microbial inactivation and the influence of different pulsed electric fields (PEF) on microbial viability was extensively studied on various microorganisms.

Since PEF microbial inactivation in controlled laboratory conditions showed promise, the idea arose of also removing pathogenic microorganisms from various water sources, hospital wastewaters and liquid food, without destroying vitamins or affecting the food's flavour, colour or texture. In order to facilitate PEF application on a large scale, the development of flow processes has been pursued. A standard PEF treatment system therefore consists of a pulse generator that enables continuous pulse treatment, flow chambers with electrodes and a fluid-handling system.

Several parameters have been described, which can influence inactivation of microbial cells. Specifically in a continuous flow system the flow rate of a liquid must be adjusted in order for each bacterial cell to be exposed to appropriate pulse conditions.

The aim of this laboratory practice is to demonstrate how different pulse parameters in a continuous flow system affect bacterial inactivation.

EXPERIMENT

We will inactivate *Escherichia coli* K12 TOP10 cells carrying plasmid pEGFP-N1, which encodes kanamycin resistance (Clontech Laboratories Inc., Mountain View, CA, USA) in a continuous flow system (see Figure 1) using different electric pulse parameters. To generate electric pulses square wave prototype pulse generator will be used. Pulses will be monitored on osciloscope (LeCroy 9310C). The inactivation level will be determined by plate count method.

Bacterial cells will be grown prior experiment for 17 hours at 37°C in Luria Broth (LB) medium (Sigma-Aldrich Chemie GmbH, Deisenhofen, Germany) with shaking.

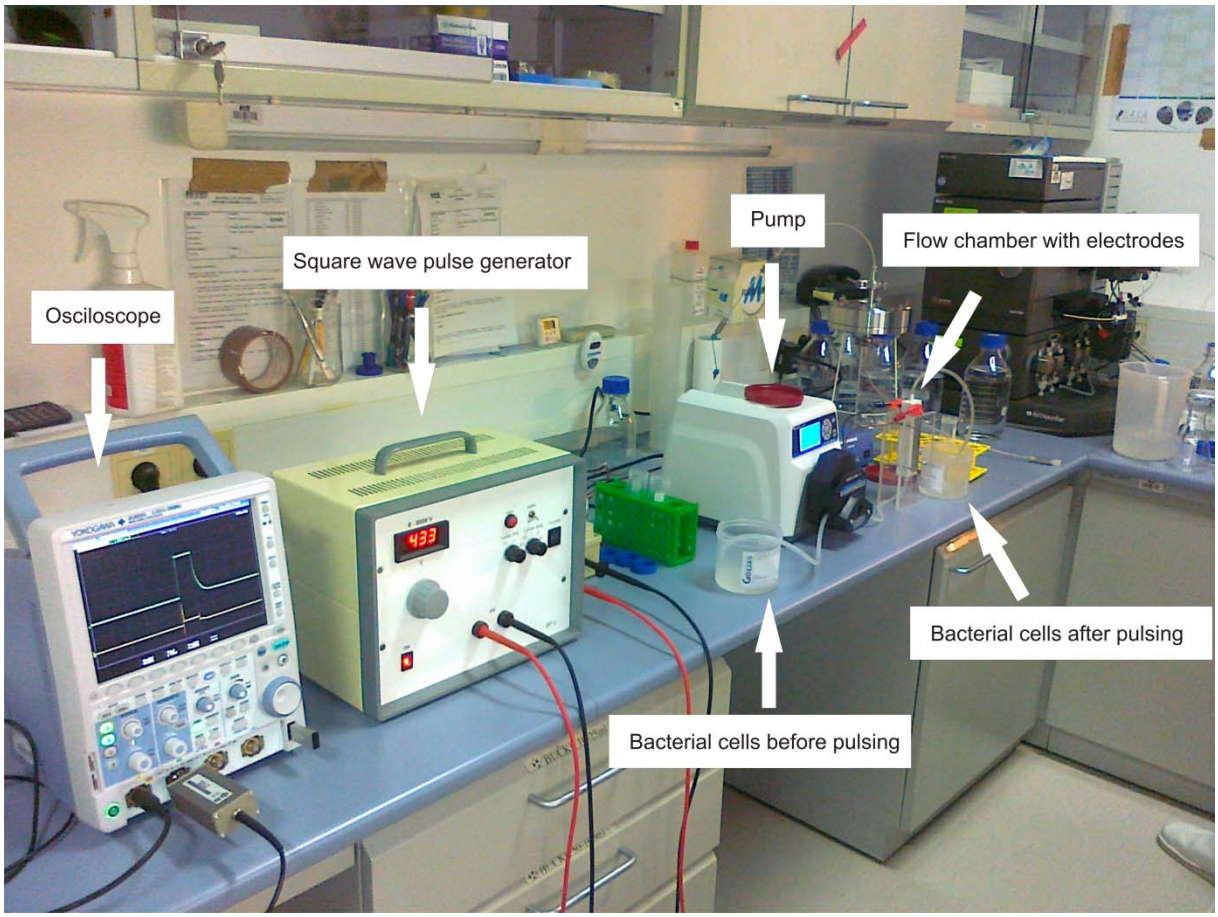

Figure 1. Continuous flow electroporation system. The circuit system includes a flow chamber with electrodes and a prototype square wave pulse generator. Voltage and current are both monitored throughout the experiment.

Protocol 1/2 (Electroporation of bacteria in a continuous flow system): On the first day of experiment *E. coli* cells will be centrifuged (4248 g, 30 min, 4°C) and the pellet will be re-suspended in 10 ml of distilled water and 100x diluted. The total volume of prepared bacterial cells for the treatment will be 0.3 L.

In order to determine the number of bacterial cells in our sample, you will prepare serial dilutions of bacterial sample ranging from 10^{-1} to 10^{-6} (in 900 µl of sterile distilled water you will dilute 100 µl of bacterial sample). You will pipette 100 µl of dilutions 10^{-5} and 10^{-6} on LB agar containing kanamycin antibiotic and evenly spread the liquid with sterile plastic rod.

The flow through the chamber in a continuous flow system depends on the geometry of the chamber, the frequency of pulses with which electroporator operates and the number of pulses and is given by equation 1. At that flow, the desired number of pulses are applied to the liquid and thus to the cells in the flow-through chamber. Because the volume of our cross-field chamber between the electrodes (Q = 0.0005 L) and the frequency (10 Hz in our case) are constant, the flow through the chamber can be determined:

$$q = \frac{f}{n} \cdot Q \qquad (1)$$

where q (L/min) is the flow rate, Q (L) the volume between the two electrodes and n is the number of pulses received by the fluid in the chamber in residence time. For a frequency of 10 Hz, you will

calculate the flow rate (*q*) at which the whole liquid will be subjected to at least one pulse. For PEF flow through treatment you will use 0.3 L (10^{-2} dilution) of prepared bacterial cells. Bacterial cells will be pumped through the system with an appropriate flow rate and pulses will be applied by square wave prototype pulse generator.

After pulsing take 100 µl of treated sample and prepare dilutions ranging from 10^{-1} to 10^{-6}. You will pipette 100 µl of dilutions 10^{-4}, 10^{-5} and 10^{-6} on LB agar with kanamycin antibiotic and evenly spread the liquid with sterile plastic rod.

Protocol 2/2 (Determining bacterial viability): After 24 h incubation at 37ºC count colony forming units. Express the viability as log (N/N_0), where N represents the number of colony forming units per ml in a treated sample (bacterial cells exposed to electric pulses) and N_0 the number of colony forming units per ml in an untreated sample (bacterial cells not exposed to electric pulses).

Example of determining bacterial viability:
You counted 70 CFU in a control sample (dilution 10^{-7}) and 30 CFU in a treated sample (dilution 10^{-5}).

Number of bacterial cells per ml (control sample) = 70 x 10^7 (dilution factor of sample) x 10 (dilution factor of plating) = 7 x 10^9 bacterial cells/ml

Number of bacterial cells per ml (treated sample) = 30 x 10^5 (dilution factor of sample) x 10 (dilution factor of plating) = 3 x 10^7 bacterial cells/ml

log N/N_0 = log (3 x 10^7/ 7 x 10^9) = ***-2.368***

FURTHER READING:

Flisar K., Haberl Meglic S., Morelj J., Golob J., Miklavčič D. Testing a prototype pulse generator for a continuous flow system and its use for E. coli inactivation and microalgae lipid extraction. *Bioelectrochemistry* doi: 10.1016/j.bioelechem.2014.03.008, 2014
Gerlach D., Alleborn N., Baars A., Delgado A., Moritz J., Knorr D. Numerical simulations of pulsed electric fields for food preservation: A review. *Innov Food Sci Emerg Technol* 9: 408-417, 2008
Gusbeth C., Frey W., Volkmann H., Schwartz T., Bluhm H. Pulsed electric field treatment for bacteria reduction and its impact on hospital wastewater. *Chemosphere* 75: 228-233, 2009
Pataro G., Senatore B., Donsi G., Ferrari G. Effect of electric and flow parameters on PEF treatment efficiency. *J Food Eng* 105: 79-88, 2011

NOTES & RESULTS

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