L6

E. coli inactivation by pulsed electric fields in a continuous mode

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Duration of the experiment: day 1: 90 min; day 2: 30 min
Max. number of participants: 4
Location: Microbiological laboratory 1
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.

The aim of this laboratory practice is to inactivate with electroporation in a flow-through system.

THEORETICAL BACKGROUND

Electroporation (under this name) was first described 50 years ago [1] and causes transient increase in permeability of the cell membrane by applying high-voltage electric field pulses. At stronger electric fields, cells are damaged, leading to cell death. Such application also known as irreversible electroporation has been previously used to inactivate bacteria in water environment [2]. The method gained ground as a tool for microbial inactivation and the influence of different electroporation parameters on microbial viability was extensively studied on various microorganisms [3].
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 [4-6]. To enable electroporation on a large scale, the development of flow-through processes has been proposed [7]. Thus, a flow-through treatment system consists of a pulse generator that provides continuous pulse treatment, flow-through 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 for each bacterial cell to be exposed to appropriate pulse conditions [8].

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 various electrical pulse parameters.

A prototype square wave pulse generator will be used to generate electrical pulses. The pulses will be monitored using an oscilloscope (LeCroy 9310C). The degree of inactivation will be determined using the plate counting method.

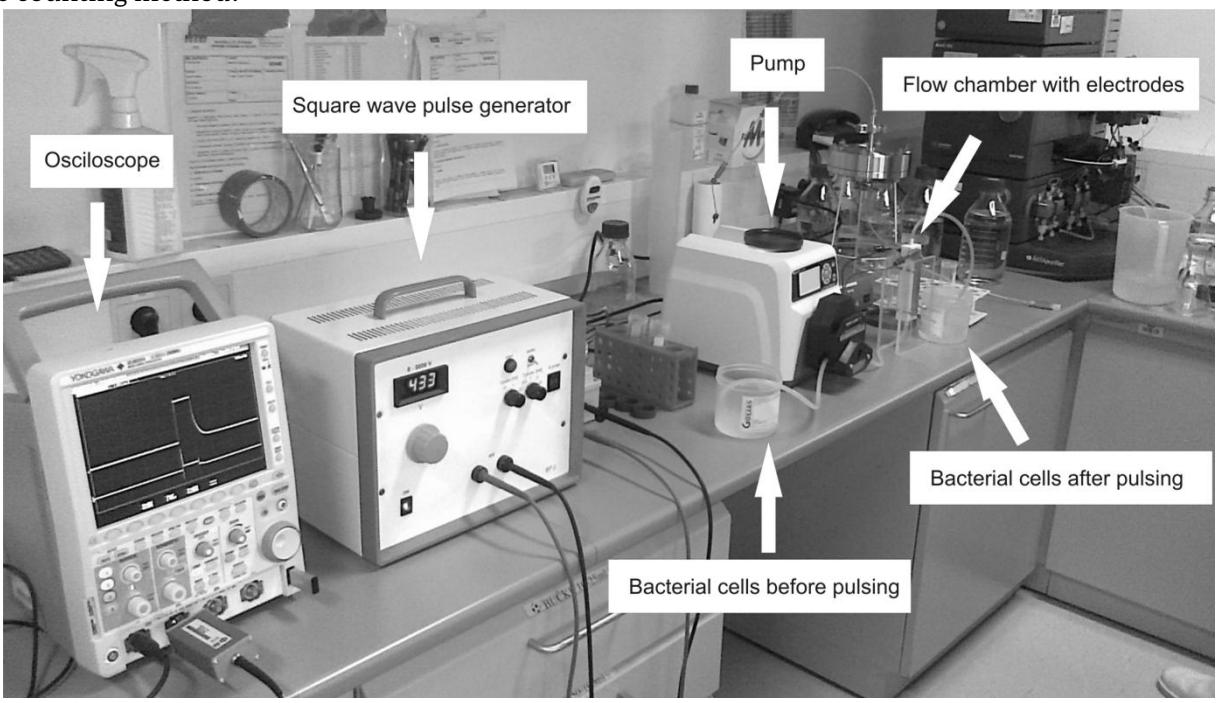

Figure 1. Continuous flow electroporation system. The circuit system includes a flow chamber with electrodes and a prototype square wave pulse generator. Voltage and current are both monitored throughout the experiment.

Protocol 1/2 (Electroporation of bacteria in a continuous flow system): 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. *E. coli* cells will be then centrifuged (4248 g, 30 min, 4°C) and the pellet will be resuspended in 250 mM sucrose.

The exposure of cells to electrical pulses in a flow chamber in a continuous flow system depends on the geometry of the chamber and the frequency of the pulses at which the electroporator operates. The number of pulses is determined by equation 1. At this flow rate, the desired number of pulses is applied to the fluid and thus to the cells in the flow chamber. Since the volume of our cross-field chamber between the electrodes and the frequency 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) is the volume between the two electrodes, and n is the number of pulses received by the fluid in the chamber during the dwell time. For a frequency of 10 Hz, calculate the flow rate (q) at which all the fluid is exposed to at least one pulse. The bacterial cells will be pumped through the system at the calculated flow rate, and pulses will be applied by the prototype pulse generator.

After electroporation treatment, collect 20 µl of the treated sample and prepare dilutions ranging from 10^{-1} to 10^{-6}. Pipette three 10-µl drops of the different dilutions onto LB agar.

To determine the number of bacterial cells in our sample, make serial dilutions of the (untreated) bacterial sample ranging from 10^{-1} to 10^{-7} (dilute 20 µl of the untreated bacterial sample in 180 µl of 0.9% NaCl). Pipette three 10-µl drops of dilutions 10^{-5}, 10^{-6} and 10^{-7} onto LB agar.

Protocol 2/2 (Determining bacterial viability): After 24 hours of incubation at 37°C, count the colony forming units. Viability is expressed as log (N/N_0), where N is the number of colony forming units per ml in a treated sample and N_0 is the number of colony forming units per ml in an untreated sample.

Example of determining bacterial viability:
You counted 20 CFU in a control sample (dilution 10^{-7}) and 10 CFU in a treated sample (dilution 10^{-5}).

Number of bacterial cells per ml (control sample) = 20 x 10^7 (dilution factor of sample) x 100 (dilution factor of plating) = 2 x 10^{10} bacterial cells/ml

Number of bacterial cells per ml (treated sample) = 10 x 10^5 (dilution factor of sample) x 100 (dilution factor of plating) = 1 x 10^8 bacterial cells/ml

log N/N_0 = log (1 x 10^8 / 2 x 10^{10}) = ***-2.301***

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NOTES & RESULTS