Bioelectrochemistry 103 (2015) 65–73

Contents lists available at ScienceDirect

Bioelectrochemistry

journal homepage: www.elsevier.com/locate/bioelechem

E. coli electroeradication on a closed loop circuit by using milli-, micro- and nanosecond pulsed electric fields: Comparison between energy costs

Alexis Guionet [a,b,c], Fabienne David [c], Clément Zaepffel [d], Mathilde Coustets [b], Karim Helmi [c], Cyril Cheype [e], Denis Packan [d], Jean-Pierre Garnier [e], Vincent Blanckaert [f], Justin Teissié [a,b,*]

[a] CNRS, IPBS (Institut de Pharmacologie et de Biologie Structurale), 205 route de Narbonne, BP64182, F-31077 Toulouse, France
[b] Université de Toulouse, UPS, IPBS, F-31077 Toulouse, France
[c] VERI, Chemin de la Digue, 78600 Maisons Laffitte, France
[d] ONERA, DMPH, Chemin de la Hunière, 91761 Palaiseau, France
[e] CERPEM, Maison de la Technopole, 6 rue Léonard de Vinci, F-53000 Laval, France
[f] MicroMar, Mer Molécules Santé, IUML-FR 3473 CNRS, Université du Maine, IUT de Laval, Dept. Génie Biologique, BP2045, 53020 Laval Cedex 9, France

ARTICLE INFO

Article history:
Received 17 February 2014
Received in revised form 25 July 2014
Accepted 12 August 2014
Available online 28 August 2014

Keywords:
Nanosecond pulsed electric field
Microsecond pulsed electric field
Bacterial inactivation
Energy consumption
Flow rate

ABSTRACT

One of the different ways to eradicate microorganisms, and particularly bacteria that might have an impact on health consists in the delivery of pulsed electric fields (PEFs). The technologies of millisecond (ms) or microsecond (μs) PEF are still well known and used for instance in the process of fruit juice sterilization. However, this concept is costly in terms of delivered energy which might be too expensive for some other industrial processes.

Nanosecond pulsed electric fields (nsPEFs) might be an alternative at least for lower energetic cost. However, only few insights were available and stipulate a gain in cost and in efficiency as well. Using *Escherichia coli*, the impact of frequency and low rate on eradication and energy consumption by msPEF, μsPEF and nsPEF have been studied and compared. While a 1 $\log_{10}$ was reached with an energy cost of 100 and 158 kJ/L with micro- and millisecond PEFs respectively, nsPEF reached the reduction for similar energy consumption. The best condition was obtained for a 1 $\log_{10}$ deactivation in 0.5 h, for energy consumption of 143 kJ/L corresponding to 0.04 W·h when the field was around 100 kV/cm. Improvement can also be expected by producing a generator capable to increase the electric field.

1. Introduction

Pulsed electric fields have been shown to affect bacterial viability [1] and are now proposed in liquid food processes to destroy microorganisms with no deleterious thermal effects [2]. This had been shown for milk [3], fruit juice [4,5] and wine [6] decontamination [7]. The mechanisms by which the pulsed electric field (PEF) with duration longer than microseconds (μs) affects the membrane organization are proposed to affect the lipid bilayer [8]. A theoretical explanation has been proposed for μsPEF where the formation of conducting defects is dependent on external field induced transmembrane potential modulation and therefore from the applied electric field amplitude [9]. Consequently, it appeared that these technologies using tens of kV/cm electric field amplitudes were effective in terms of decontamination for the food processing. One open question was that pulses with μs duration period were associated with high energy consumptions. New pulse generators provide a lot of flexibility in the choice of pulse duration, rise time, voltage, frequency and time of treatment. They therefore give access to a systematic investigation of the optimized conditions for a minimized energy consumption. A key observation is that energy consumption was not directly proportional to cell inactivation. This concept has been shown to be valid on *Escherichia coli* with pulsed electric fields using 2 different pulse durations at 32 ns and 700 ns; eradication levels were different while energy consumption used was equivalent [10].

A societal problem is to obtain a safe and “green” method to keep safe water in large volume reservoirs. PEF is clearly a solution. For technical reasons, a batch process is not conceivable and a flow process on a derivation can be a suitable method (Fig. 1).

In the resulting closed flow system, eradication depends on the electrical parameters, the flow rate, the volume of the decontamination chamber and the treated volume of the tank (reservoir). Trains of pulses are delivered when the bacteria are in the applicator. The technology is controlling the number of pulses that are delivered on the sample and the delay between the delivery of pulse trains on a given bacteria. This last parameter was shown to be important on the effect of PEF [11,12]. Increasing the flow rate will increase the number of passages where a

* Corresponding author at: CNRS, IPBS (Institut de Pharmacologie et de Biologie Structurale), 205 route de Narbonne, BP64182, F-31077 Toulouse, France.
E-mail address: Justin.teissie@ipbs.fr (J. Teissié).

http://dx.doi.org/10.1016/j.bioelechem.2014.08.021
1567-5394/

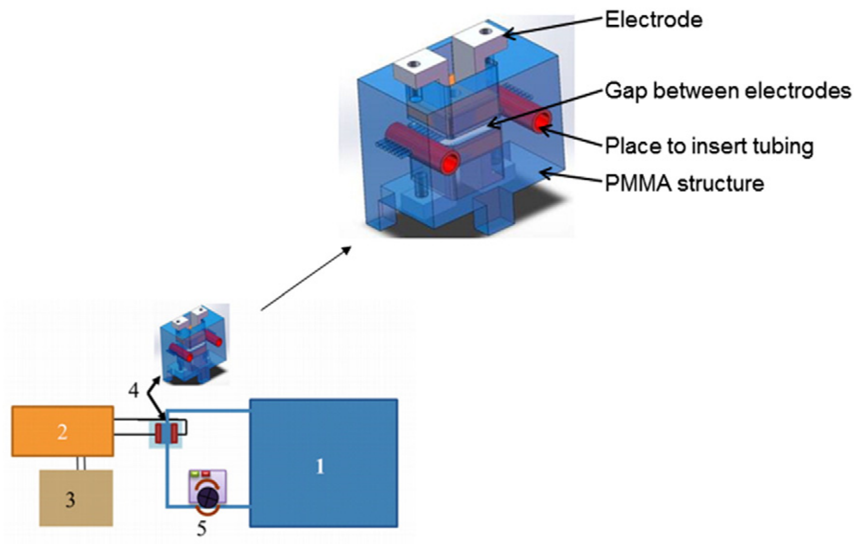

Fig. 1. Schematic drawing of a flow system with a derivation in order to destroy the bacteria. 1. Volume of water to be treated, 2. Generator, 3. TTL, 4. Flow disinfection chamber or flow applicator, 5. Peristaltic pump. Arrows on the peristaltic pump show the fluid travel in a clockwise direction. In the upper right corner: Applicator or treatment chamber with a gap between electrodes of 2 mm. The generators after pulse deliveries induced in the chamber a homogeneous electric field of 100 kV/cm.

cell is treated, but it decreases its residency per passage in the decontamination chamber, i.e. number of pulses that are applied.

In this study, several sequences of pulsed electric fields have been investigated: nsPEF, μsPEF and msPEF with several frequency and flow conditions. From a more applied point of view, we took into account that the level of contamination is monitored in a quasi-continuous way. Bacteria contamination results not from the growth in the liquid phase but from the sporadic release from the biofilms present on the wall of the reservoir. Electrically mediated decontamination should act as soon as a small contamination is detected and a 1 $\log_{10}$ eradication is therefore just what is needed. From a basic point of view, μsPEF and msPEF were assumed to act by a membrane irreversible permeabilization while nsPEFs were predicted to act by the same way but in synergy with a direct effect on the cytoplasmic content [13,14]. The energetic consumptions needed for a 1 $\log_{10}$ eradication after the millisecond, the microsecond or the nanosecond treatments have been compared.

2. Materials and methods

2.1. Microbiological culture and sample preparation

Experimental studies were performed by using *E. coli* BL21(DE3) grown in LB broth and throughout called *E. coli*. They were stored at 4 °C as "colony forming unit" (CFU) on Petri dish filled with "Plate count agar" PCA. A CFU was incubated at 37 °C under agitation in 60 mL of culture medium. After 15 h the bacteria population reached a stationary phase. Germs were harvested by centrifugation (3000 g, 10 min). The supernatant was discarded and the pellet resuspended in a Tris saline buffer (pH 9) after discarding supernatant and vortexing. The final concentration of *E. coli* in solution was 10^9 CFU/mL.

2.2. Pulsation solution

It was prepared by adding in ultra-pure water, ions at the same concentration than those measured in an industrial water. The pH was buffered with Tris and adjusted at pH = 9 (as found in industrial water) by NaOH or HCl. Conductivity was adjusted at 2.5 mS/cm using NaCl. Stability of pH, conductivity, and bacteria concentration were compared between water from industrial water and the Tris solution. No significant difference was observed in survival over 24 h.

2.3. Nanosecond batch or flow electropulsation assays

2.3.1. Generator characteristics

Two different pulse generators were used.

2.3.2. Generator characteristics

Two pulse generators were built by ONERA and used for this study. The aim of both generators is to apply an electric field of 100 kV/cm to the applicator, with an applicator gap of 2 mm, this correspond to a voltage of 20 kV. However, generator G2 operates within a different frequency range.

2.3.2.1. Generator G0. The first generator delivers square pulses of 70 ns, 20 kV at 2 Hz on an 80 Ω impedance corresponding to the reactor impedance. The principle is a 12-meter coaxial transmission line being charged by a high voltage DC power supply and then discharged to the applicator. The discharge of the transmission line is triggered using a commercial spark gap (GP-12B Excelitas) giving a pulse rising time of 20 ns. In an impedance matched case (impedance of the load equal to the impedance of the line), the voltage across the reactor should be half the transmission line charging voltage. Here we use a high impedance reactor (80 Ω) and avoid matching impedance to double this voltage thus reaching a voltage across the reactor equal to the charging voltage. The downside is the propagation of a reflected pulse toward the generator. A 12 Ω resistance is thus used to absorb this reflected pulse, this limits the maximum frequency of the generator. The pulse duration is directly correlated to the propagation inside the 12 m transmission line resulting in a pulse duration of 70 ns. A P6015 high voltage probe from Tektronix is used to monitor the high voltage on the applicator. A pulse profile of the G0 is presented Fig. 2.

2.3.2.2. Generator G2. The second generator was designed to deliver the same electric field of 100 kV/cm but at higher frequency up to 20 Hz and with alternating bipolar pulses to avoid electrolysis. As in the first generator, a 12-meter coaxial transmission line was used, that defined the pulse duration of 70 ns. The DC commercial power supply was replaced by a pulse transformer charging circuit able to deliver successively positive and negative high voltage pulses of 2 μs charging the transmission line, a timing of 50 ms separated both pulses. To achieve this, two primary circuits were wired around the transformer, one for each polarity.