Fast flow-through non-thermal pasteurization using constant radiofrequency electric fields

Jarold González-Sosa; Albert Ruiz; Guillem Arias; Quim Castellví*; Antoni Ivorra

antoni.ivorra@upf.edu Departament de Tecnologies de la Informació i les Comunicacions, Universitat Pompeu Fabra, Barcelona, Spain

Abstract

Pulsed Electric Field technologies have captured the attention of researchers on food pasteurization because of their non-thermal inactivation mechanism, which results in fresh-like products. Nevertheless, high voltage pulsing required by these technologies implies complex and costly generators. Here, as an alternative, it is proposed a method, partially inherited from research on cell electroporation for gene transfection, in which the liquid to be treated flows at high speed through a miniature chamber where the electric field is permanently applied. In particular, it is proposed that the constantly applied electric field consists of an AC signal (> 100 kHz) so that electrochemical by-products are minimized. The method, while being compatible with batch processing, will allow use of lower voltages and will avoid the pulsation requirement.

Background

Thermal methods are the basis of most pasteurization technologies intended for food preservation. High temperatures, however, not only inactivate harmful microorganisms but also damage constituents of the medium under treatment, which may result in detrimental effects on nutrients, color, flavor and texture.

In the last decades, **Pulsed Electric Field (PEF) technologies** have captured the attention of researchers because:

- Growing demand for fresh-like foods.
- PEF technologies have the potential to minimize energy consumption.
- PEF technologies could be relevant in the context of renewable resources (for efficient oil extraction from microalgae for biofuel production).

Nevertheless, PEF methods have some disadvantages that hinder their industrial adoption. Among these disadvantages:

- High maintenance costs due to electrode erosion.
- High cost of electrical pulse generators.

Cell killing action in PEF technologies is based on the phenomenon known as electroporation which causes cell membrane permeability to increase when the cell is briefly exposed to a high electric field. In current PEF technologies, the liquid to be treated is forced to flow at moderate speed through a relatively long chamber containing electrodes between which a number of high voltage pulses are applied so that the required high electric fields for electroporation are produced. Pulses have to be of short duration (<1 µs) in order to avoid heating. This is technically feasible but it comes at an economic cost because it requires sophisticated high power switches.

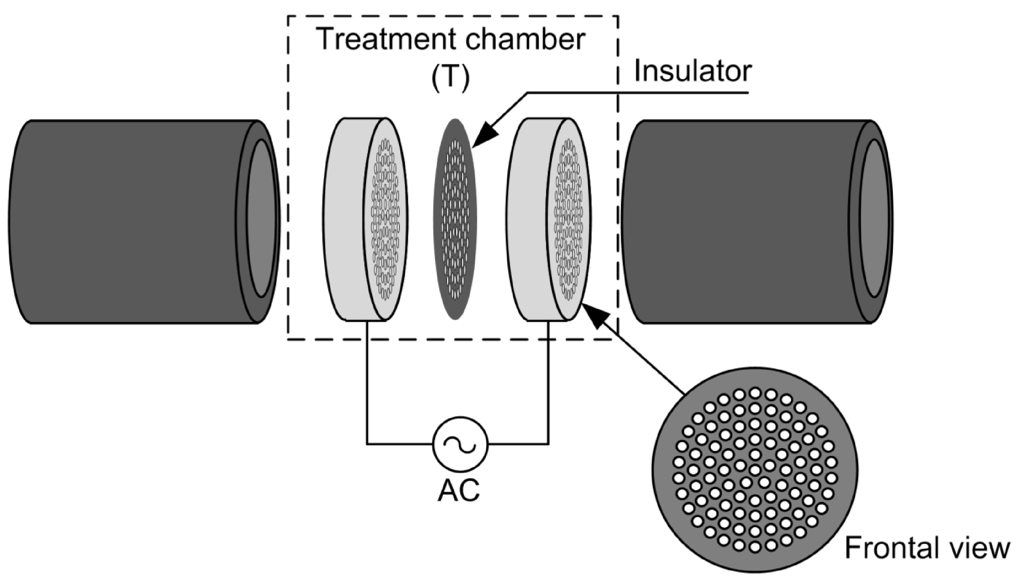
The proposal

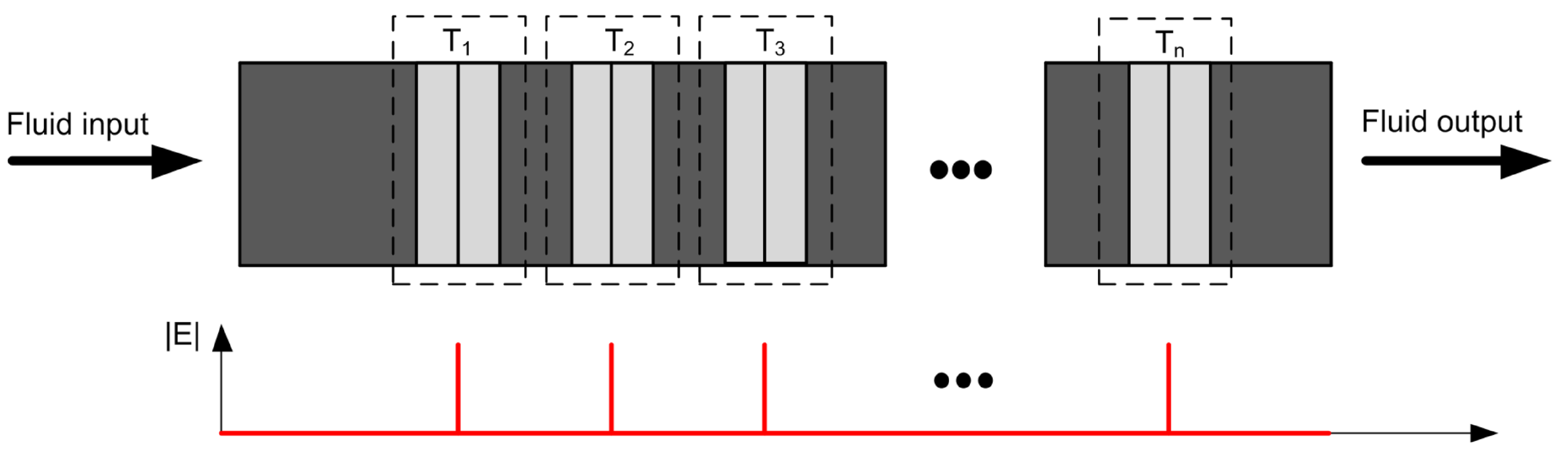
Electroporation is employed in microbiology laboratories for gene transfection. Recently, in order to transfect large quantities of cells, it has been proposed to electroporate the cells by making them flow at high speed through a microfluidic chamber where the electric field is permanently applied (Nat Protoc. 2011; 6(8):1192-208). This concept is usually referred to as **flow-through electroporation** and it:

1. Does not require ultra high voltages. In a small chamber the electric fields (i.e. voltage gradients) can be made very large with lower voltages.

2. Does not require voltage pulsation as pulsation intrinsically occurs.

Here we show that this concept is applicable to non-thermal pasteurization despite that much higher fields are required for killing cells than for transfection. In addition, we propose to employ constant radiofrequency voltages (>100 kHz) so that electrochemical reactions at the electrodes are minimized.



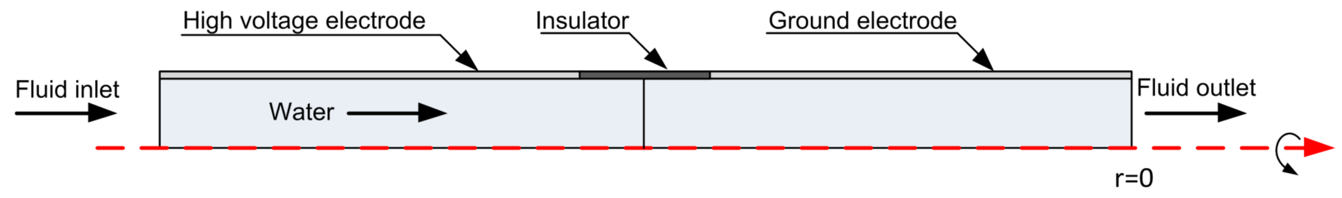


Proposed implementation of the flow-through electroporation concept for industrial pasteurization: the fluid to be treated would flow at high speed through orifices in a three-layer structure consisting of a thick metallic electrode, a thin insulator and a second thick metallic electrode. The electrodes would be energized by a moderate AC voltage so that **each portion of fluid would experience a high electric field AC burst during passage through the insulator**. Concatenation of treatment chambers is proposed for higher effectiveness.

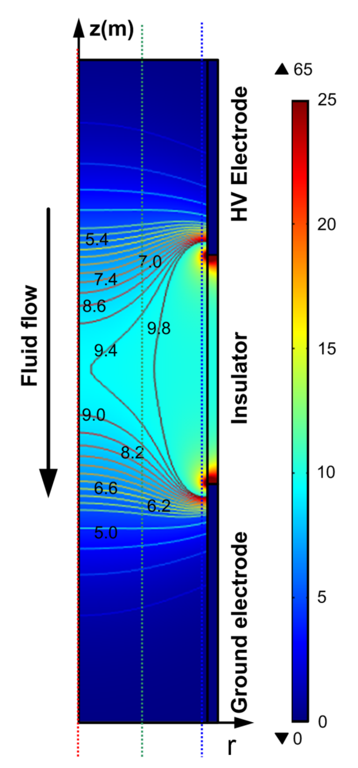
Numerical study

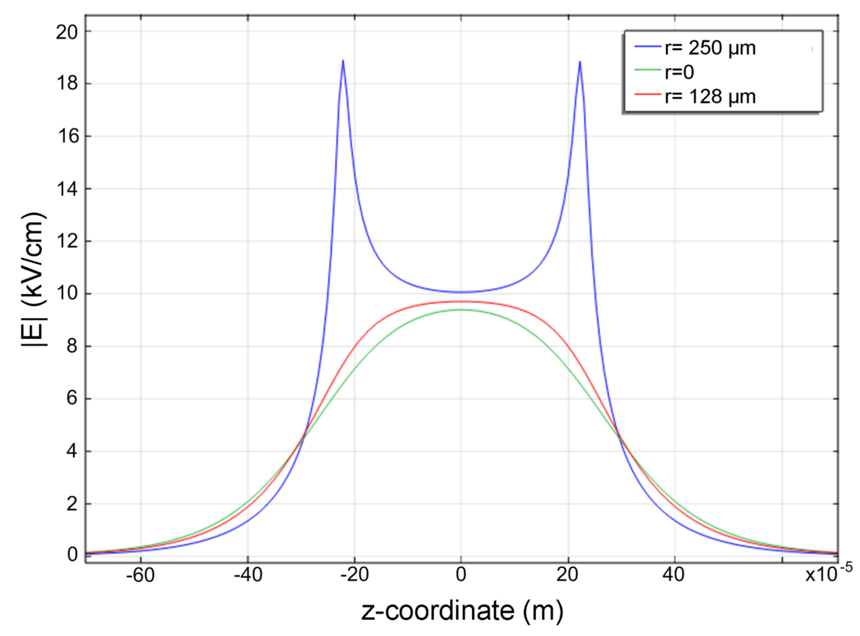
A model was designed for simulating a single conduit of the proposed three-layer structure. The results show that, in an scenario where feasible fluid speed is forced and high electric fields capable of killing bacteria are produced, mild temperature increase is induced.

The model consisted of slightly conductive water flowing through an insulator tube in between two metallic tubes. Dimensions and other parameters were selected as to be consistent with the in vitro study later described (total length = 40 mm; insulator length = 0.45 mm, inner diameter = 0.5 mm). It was simulated in COMSOL Multiphysics 4.2a, which is a numerical software platform able to concurrently model diverse physics. In this case, three physics were modeled: **DC electrical conduction, turbulent flow** (standard k-epsilon model) **and heat transfer**. The applied DC voltage was 570 V which corresponds to the RMS value of the applied bipolar square pulses in the in vitro study. Water input speed was imposed to be 5 m/s. Axial symmetry was used for minimizing computation time.

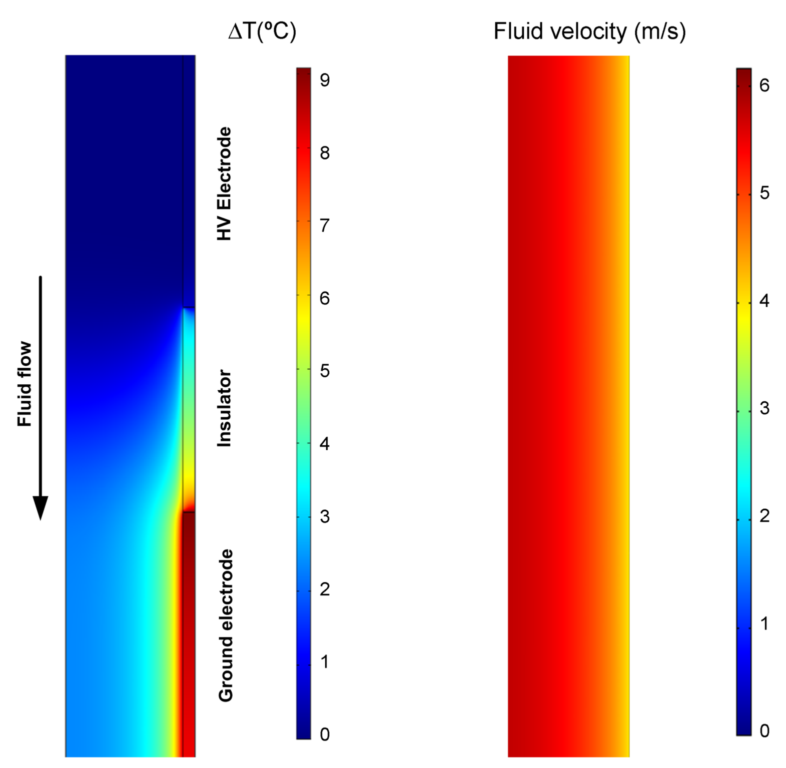


	Stainless steel	PTFE	Conductive water
Thermal conductivity, k ($W \cdot m^{-1} \cdot K^{-1}$)	44.5	0.24	0.602
Mass density, ρ ($kg \cdot m^{-3}$)	7850	2200	998
Heat capacity, C_p ($J \cdot kg^{-1} \cdot K^{-1}$)	475	1050	4183
Electrical conductivity, σ ($S \cdot m^{-1}$)	4.032×10^{6}	1×10^{-25}	0.15
Dynamic viscosity, μ ($Pa \cdot s$)	–	–	0.89





⇧ Electric field magnitude (RMS). The edge effect is noticeable; the maximum electric field magnitude is experienced at the center and it is about 9 kV/cm whereas the electric field close to the surface reaches peak values well above 15 kV/cm. This electric field heterogeneity is not relevant provided that all fluid portions are sufficiently treated and that no fluid portion is excessively heated for too long. Total current flowing through the chamber is 30 mA (RMS) which implies that the chamber resistance is 19 kΩ.



⇦ Fluid temperature and velocity. Because of lower fluid velocity in the vicinity of the tube wall and because of the field edge effects, which cause heating spots, temperature increase is particularly significant on the tube inner surface. Nevertheless, such local temperature increase is below 9 ºC and it is rapidly diluted downstream. The average temperature increase at the exit of the treatment chamber is 3.4 ºC.

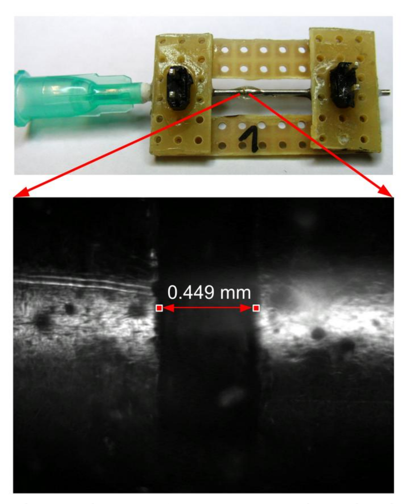
In vitro study

A proof-of-concept system was built for demonstrating effectiveness of the fast flow-through non-thermal technique proposed here for killing E. coli bacteria.

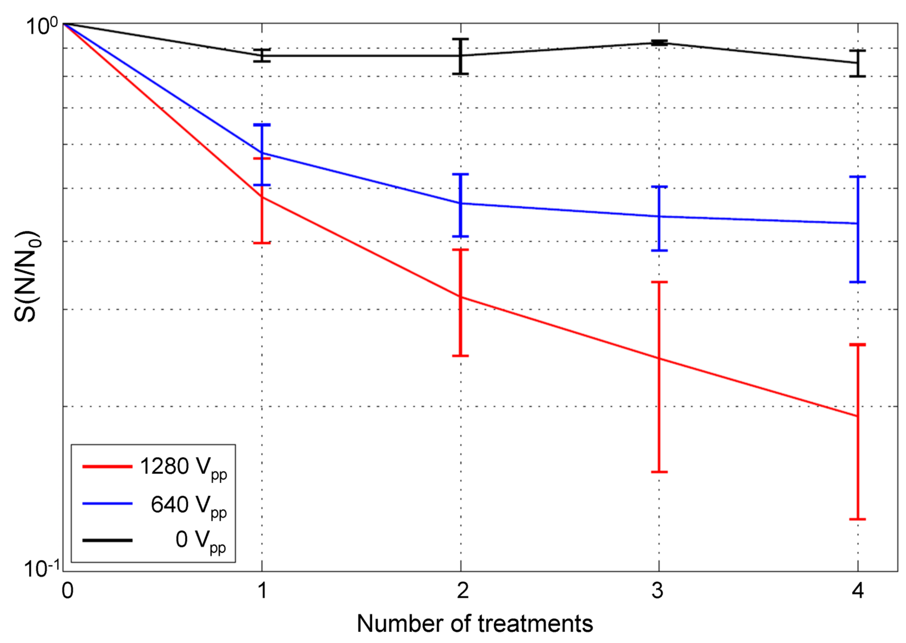
The system consisted of a high voltage generator, a treatment chamber and a syringe pump. The generator delivered bipolar square signals at 100 kHz with a peak to peak amplitude of up to 1280 V. The treatment chamber was made from a stainless steel hypodermic needle (gauge 21): the needle was cross cut into two sections and a dielectric epoxy conduit with a length of 0.45 mm was created. The custom made syringe pump was set so that the water velocity through the chamber was 5 m/s.

Treated samples consisted of mineral water intentionally contaminated with Escherichia coli (strain ATCC # 25922): bacterial culture in LB broth was 1/20 diluted in the water. Electrical conductivity of the samples was approximately 0.15 S/m and concentration of bacteria was about 3×10^{8} CFU/ml. Counting was performed measuring viable plate counts after 48 hours incubation on MacConkey's agar.

In order to emulate concatenated chambers, experiments were performed in which the treated water samples were treated again up to three times more.



(experimental treatment chamber)



(error bars correspond to the maximum and minimum measured values in three repetitions)

⇦ Cell counting results. The method is indeed capable of performing bacteria inactivation but, for industrial applications, higher fields (or more treatments) will be required because the obtained CFU reductions were low.

Fluid temperature was measured after treatment when 1280 V_{pp} were delivered and it resulted in an average temperature increase of 4.5 ºC. By performing dummy treatments (0 V) the average temperature increase was about 0.5 ºC. That is, of the 4.5 ºC increase during treatment about 0.5 ºC correspond to friction rather than to Joule heating.

Treated samples were checked for iron contents, by means of test strips which have a detection sensitivity of 0.1 ppm, with negative results.

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