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Testing a prototype pulse generator for a continuous flow system and its use for *E. coli* inactivation and microalgae lipid extraction

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ABSTRACT

Among other applications, electroporation is used for the inactivation of pathogens and extraction of substances from microorganisms in liquids where large scale flow systems are used. The aim of our work was therefore to test a pulse generator that enables continuous pulsed electric field (PEF) treatment for *Escherichia coli* inactivation and microalgae lipid extraction.

In the continuous flow PEF system, the flow rate was adjusted so that each bacterial cell received a defined number of pulses. The results of PEF flow treatment showed that the number of pulses influences *E. coli* inactivation to the same extent as in the previously described cuvette system, i.e., batch system.

The continuous flow PEF system was also tested and evaluated for lipid extraction from microalgae *Chlorella vulgaris*. In control experiments, lipids were extracted via concentration of biomass, drying and cell rupture using pressure or an organic solvent. In contrast, electroporation bypasses all stages, since cells were directly ruptured in the broth and the oil that floated on the broth was skimmed off. The initial experiments showed a 50% oil yield using the electroporation flow system in comparison to extraction with organic solvent.

1. Introduction

The first description of the profound effects of electrical pulses on the viability of a biological cell was given in 1958 [1] and a decade later, Neumann et al. showed that electric pulses cause transient permeability changes in the membrane of a vesicle [2]. The field expanded greatly [3] and it was shown that if the electric field applied to a biological cell is sufficiently intense, the cell loses its homeostasis and eventually dies — irreversible electroporation [4]. The method gained ground as a tool for microbial inactivation [5,6] and the influence of pulsed electric fields (PEFs) on microbial viability was extensively studied on various Gram-positive bacteria [7,8], Gram-negative bacteria [9–11], yeasts [12], protozoa parasites [13] and even spores [14]. Since PEF microbial inactivation in controlled laboratory conditions showed promise, the idea arose of also removing pathogenic microbial agents from various water sources [15–19] and from liquid food, without destroying vitamins or affecting the food's flavor, color or texture [8,20–26].

Electroporation has also been used to extract molecules from cells [27], i.e., proteins from various microorganisms [28–31], plasmid DNA from bacterial cells [11,32], sugar from sugar beet cells [33–35] and oil for biodiesel from oil-producing microalgae [31,36–46]. Microalgae offer great potential in biodiesel production in comparison to production from lipids out of farm crops (soy bean, corn, and grape) due to their fast growth and non-demanding environment suitable for their growth, which enables microalgae production away from fertile farm land and in waste water treatment plants. The lipid content of microalgae is high — up to 50% of the cell dry weight. Because of their small size (around 10 μm), their surface area is large, which enables a higher CO_2 absorption rate and, consequently, better photosynthesis efficiency [42,43].

In order to facilitate PEF application on a large scale, the development of flow processes has been pursued [47]. 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 [48]. Electroporators designed for PEF flow application must meet specific and often demanding requirements: high voltage and high current pulses operating in flow-through systems, i.e., continuous operation. Most commercial electroporators are therefore not suitable for research on bacteria inactivation or the extraction of various substances from cells, although they have the necessary electric field strength from 10 kV/cm to over 100 kV/cm [49], with continuous operation (using flow chambers) and operation with high frequency pulses. However, large scale electroporators (PEF systems) require large treatment volumes and have fixed pulse parameters, so they are not useful for investigating the pulse parameter effect in a treatment process [50].

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The aim of our work was therefore to develop a suitable laboratory scale square wave pulse generator that enables continuous delivery of high voltage and high current electric pulses. The pulse generator was tested using two different flow-through electrode chambers in two different promising biotechnological PEF applications: irreversible bacterial inactivation and lipid extraction from microalgae.

The developed pulse generator operates over a wide range of pulse parameters, unlike industrial installations that are optimized for specific (optimal) parameters [51]. Although a Marx generator enables high output power with a C-discharge circuit, its use is more restricted due to the limited choice of parameters [52]. When using flow chambers, additional problems arise: the stability of the circuit for generating pulses and an adequate supply of energy required for a continuous operation electroporator. The electroporator that we describe and which we tested on two different flow-through treatment chambers requires a strong source (at least 1 kW), since it works continuously, and an appropriate switch for generating pulses. For a laboratory version, a square wave generator [53] is the best solution but a switch for generating pulses must be properly installed.

2. Material and methods

2.1. Square wave generator and continuous flow system

A key problem of a high voltage square wave generator is a switching element (MOSFET or IGBT transistor) that, with the current technology, allows operation with voltages of a few kV. There are a number of possible solutions for obtaining the voltages required in this kV range, including: semiconductors coupled in series, controlled by optic fiber or the use of an output transformer. The solution with an output transformer is limited by the range of the maximum current, operating frequency and pulse shape but it provides galvanic insulation. For research in the broad field of applications, the preferred choice is an electroporator built on the basis of series coupled semiconductors controlled via optic fiber, the advantages of which are a simple secure connection between the control electronics and the power semiconductors without any limitation, over any distance without inductive and capacitive effects, simple testing and low price [54–56].

When using a conventional square wave generator, a major limitation is the voltage and current capacity of solid state switches. Switching to voltages of a few kV, despite the advances in the semiconductor technology, is still proving to be problematic. In a series configuration, the load voltage of semiconductors can be reduced but problems can occur because the wiring introduces additional inductance and capacitance in the circuit, which results in voltage spikes [57]. With the development of semiconductor devices such as high-voltage bipolar and MOSFET transistors, IGBTs and the introduction of semiconductors based on SiC and GaN technology, the maximum operating voltage of semiconductors has further increased, while initial reports state the achievement of several thousand amperes [58,59]. When the switching elements are connected in series, the major problem is the stress on each switching element because of the power distribution in the semiconductors, which should in most cases be multi-level, i.e., floating [60]. The stress distribution depends primarily on the characteristics of the semiconductors, which are in principle equal, although the uneven cooling of an individual semiconductor, having a positive temperature coefficient, and parasitic capacitance between the semiconductors and the heat sink can lead to uneven stress distribution [61].

Circuits for the even distribution of voltage in semiconductors connected in series more or less influence the choice of control mode of individual semiconductor devices and it is the easiest if the control semiconductors in series are electrically isolated. Moreover, snubber or active voltage balance circuits ensure an even voltage distribution in the semiconductors. For switching voltages of up to 4 kV, conventional optocouplers at high voltage can be used; for higher voltages, the choice is among the special optocouplers, optical fibers or transformers [62,63]. Transformers used for transforming alternating current input voltage into output voltage have coils with a common iron core. However, due to their dimensions, it is difficult to achieve high dielectric strength. The use of fiber-optic insulators is therefore simpler and poses virtually no restrictions on the level of galvanic insulation; however, it requires an additional power supply for the optical receiver [64].

Series-connected semiconductors are built with basic building blocks: a semiconductor driving circuit with galvanic insulation, a balance (distribution) circuit, a protection circuit and a power supply (DC/DC converter). This is actually a basic stack in series. If there is a need to build a simple switching element for switching at high voltages, in terms of the availability of elements on the market, such a basic unit should provide at least 1 kV peak voltage and, by connecting one or more stacks in series, multiple operating voltages are obtained by stacking an appropriate number of single switching units. In order to ensure the fastest switching response, the use of SiC semiconductors is recommended but their lower current-carrying capability has to be taken into account.

The second most important part of an electroporator is the voltage source, which is not strong enough in most electroporators and allows only a limited number of pulses of limited duration. We assume that by applying 5000 V, the current through the load would be 10 A. With a 1 ms pulse duration, the average power (P_{aw}) at 10 Hz frequency would be 500 W. However, it is necessary to take into account the losses in the semiconductor switch and a safety factor, so a power source up to 1000 W is needed. Conductor simulations indicate that the appropriate pulses in processing a liquid with conductivity of 12 mS, require a 1 kW power source. However, the power source must also have very good dynamics [65], which means an inverter with high frequency and the selection of full-bridge topology.

The flow through the chamber in a PEF 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 Eq. (1). At that flow, the desired number of pulses is applied to the liquid and thus to the cells in the flow-through chamber [66]. Because the volume of the chamber between the electrodes (Q) and the frequency (10 Hz in our case) are constant, the flow through the chamber (Eq. (1)) and number of pulses can be determined (Eq. (2)):

$$q = \frac{f}{n} \cdot Q \tag{1}$$

$$n = \frac{f}{q} \cdot Q \tag{2}$$

where q (L/min) is the flow through the chamber and Q (L) is 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, we calculated the flow rate (q) at which the whole liquid is subjected to at least one pulse. For a cross-field chamber with a capacity of $Q = 0.0004$ L and for a co-field chamber with a capacity of $Q = 0.0012$ L, we calculated flow rates of $q = 0.24$ L/min and $q = 0.72$ L/min, respectively. From the flow rate and quantity of liquid, we calculated the time of the experiment ($t = Qc/q$), where Qc (L) is the whole liquid prepared for the experiment. The time of the experiment (time needed for the whole liquid to be subjected to at least one pulse) was therefore approximately 3 min for 0.5 L of *Escherichia coli* (for 8 pulses, 24 min is needed for the liquid) and approximately 2.77 min for 2 L of microalgae liquid. PEF treatment time is calculated using the following equation:

$$t = n \cdot w \tag{3}$$

where n is the number of pulses and w (s) is the pulse width.

Fig. 1 shows the experimental system (laboratory scale continuous flow system) used in this study. The circuit system includes a cross-field (for bacterial cells) and co-field (for microalgae) chamber and a