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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

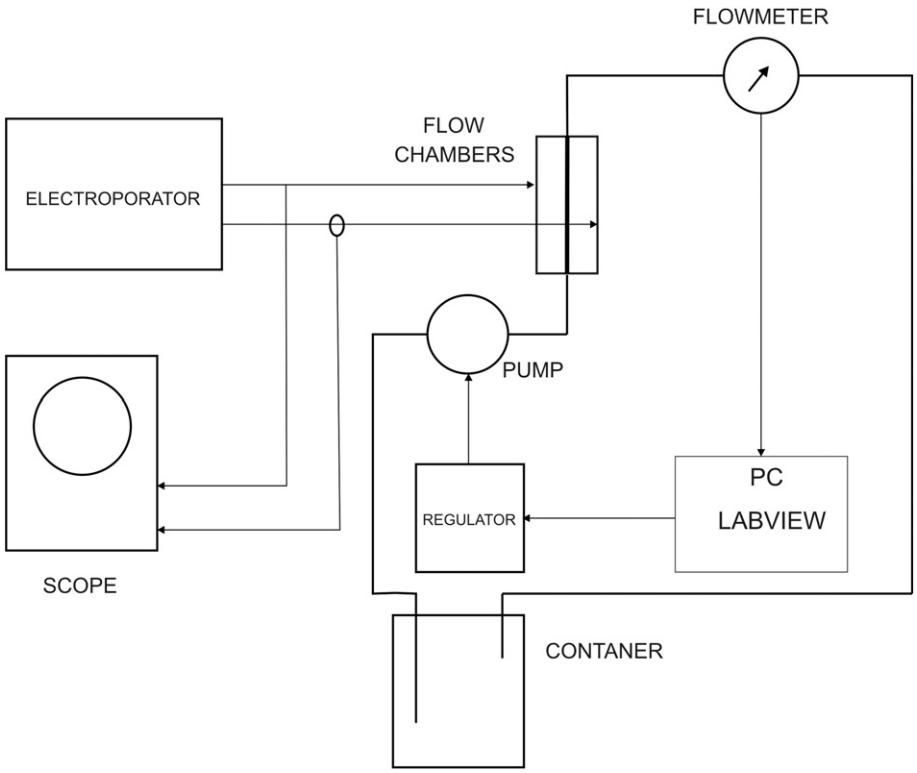

Fig. 1. The circuit system includes a cross-field (for bacterial cells) or co-field (for microalgae) chamber and a prototype square wave pulse generator. Voltage and current are both monitored throughout the experiment.

prototype square wave pulse generator, which enables continuous delivery of electric pulses. Both chambers are presented in Fig. 2.

Experiments were carried out by pumping the liquid product (bacterial cells or microalgae) through a continuous flow treatment chamber [67,68] consisting of a polytetrafluoroethylene (PTFE) insulator enclosed by high voltage and ground electrodes made of stainless steel. The chamber has an inner diameter of 25 × 25 mm (square for bacterial cells) or 10 mm (round for microalgae) and an electrode gap of 2.5 mm (for bacterial cells) or 15 mm (for microalgae) (Fig. 2).

2.2. Bacterial cells

E. coli K12 TOP10 cells carrying plasmid pEGFP-N1, which encodes kanamycin resistance (Clontech Laboratories Inc., Mountain View, CA, USA) were grown in LB Luria Broth medium (Sigma-Aldrich Chemie GmbH, Deisenhofen, Germany) with shaking. Bacterial selectivity was maintained by the addition of antibiotic kanamycin sulfate (Carl ROTH GmbH, Essen, Germany) at a concentration of 50 μg/μL. *E. coli* cells were grown for 17 h at 37 °C to a final concentration of approximately 10^9 CFU/mL (determined by the viable count method). The LB medium was removed by centrifugation (4248 g, 30 min, 4 °C) and the cells in the pellet were re-suspended in 10 mL of distilled water. We used 10^{-2} dilution of prepared bacterial cells for the experiment. The total volume of liquid for the treatment was 0.5 L.

2.3. Inactivation of bacterial cells

Bacterial cells were exposed to electric pulses using a laboratory scale continuous flow system with square wave prototype pulse generator (see square wave generator description in Material and methods). In order to check the influence of electric pulses on bacterial viability, constant electric field (E), pulse duration and frequency were used (12 kV/cm, 1 ms, 10 Hz, respectively). Bacterial cells in the cross-field chamber received one, four or eight pulses, since the treatment time of the whole liquid (0.5 L) was 3, 12 or 24 min, respectively. The applied electric field (E) is approximated by $E = U/d$, where U denotes the applied voltage and d denotes the electrode distance ($d = 2.5$ mm). It has already been shown in a cuvette system that increasing the pulse number or treatment time also increases the inactivation of bacterial cells [8,10,19,69,70]. A study on mammalian cells also showed that with increasing pulse number, the fraction of long-lived pores, as well as cell membrane permeabilization, also regularly increases [71]. We therefore decided also to test the influence of pulse number on bacterial inactivation in our continuous flow system. Since it has been shown that longer pulse duration (up to 1 ms) creates a subpopulation of large, i.e., long-lived pores on the cell membrane [72], and that any increase in pulse number or/and pulse duration tends to improve bacterial inactivation [5], we expected to achieve higher inactivation of bacterial cells when applying longer (1 ms) pulses.

Before the experiment, the flow system was washed with sterile distilled water and sterilized with 70% ethanol. After the ethanol in the system had dried, the tubes and electrodes were again rinsed with sterile distilled water. Flow processing was then applied to 0.5 L of *E. coli* cells, with a flow rate of 0.24 L/min through the chamber ($d = 2.5$ mm) and pulses were applied. The total treatment time of the sample was 3, 12 or 24 min, so each bacterial cell received one, four or eight pulses, respectively. The conductivity of the bacterial suspension was 1.2 mS/cm^2. After electric pulse application, 50 μL of sample was taken in order to determine the influence of the electric pulses on *E. coli* viability. All the experiments were performed at room temperature (22 °C). The impact of electric pulses on bacterial viability was defined by the plate count method [73]. Cells were serially diluted with distilled water and 100 μL of dilution was plated onto Luria broth agar medium (Sigma-Aldrich Chemie GmbH, Deisenhofen, Germany). Plates were incubated for 24 h at 37 °C and counted manually. The viability was expressed 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 represents the number of colony forming units per mL in an untreated sample

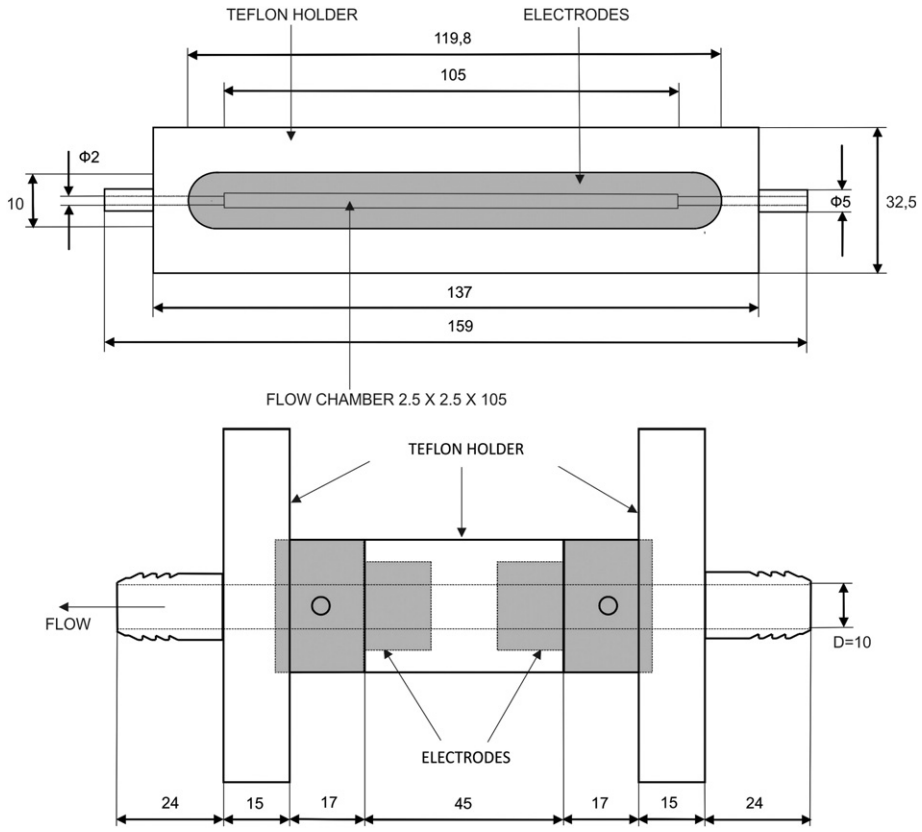

Fig. 2. Cross-field chamber (above), used for *E. coli* and co-field chamber (bottom), used for microalgae. The gray area represents metallic parts (electrodes) of the chambers.

(bacterial cells not exposed to electric pulses). Experiments were repeated three times and each data point in the results is the mean value of all three experiments, with standard deviations shown by error bars.

2.4. Microalgae treatment

Microalgae *Chlorella vulgaris* were grown for 14 days in a 60 L photo bioreactor that contained a mixing vessel (50 L) and tubes with a light supply. Algae were grown by the batch principle. The algae culture was diluted in a medium rich in raw materials (Jaworski medium) and then grown in original medium for two weeks. The microalgae were exposed to artificial light (wavelength 410–450 nm and 670–678 nm) for 16 h daily. During growth, pH and oxygen consumption were monitored automatically. pH was regulated via a computer program and regulation performed by blowing CO_2 into the reactor. The broth was aerated with 1000 L/h of air. Biomass concentration via cell number was monitored daily. After the growing procedure, and an extraction procedure using organic solvent for control, the algal broth was left to settle for a week in a refrigerator. The broth was then centrifuged and the biomass was lyophilized. Cell membranes were disrupted with acid and lipids were extracted with petroleum ether [46].

Using the flow-through system developed for this study and outlined in Section 2.1, the same broth as used in the previous test was directly pumped into the PEF continuous flow chamber with a minimum flow of 0.72 L/min, which guaranteed that no air bubbles were present in the tube during the procedure. We measured the conductivity (1 mS/cm^2) in a preliminary study to avoid electrical breakdown through the conductive broth if the applied voltage was too high. The range of applicable voltages was subsequently determined. The average cell size was measured with image processing software integrated into an optical microscope (NIS elements BR3.10, NIKON). The size of cells after 14 days growth was 3.8 $\pm$ 1.2 μm.

The broth was pumped through the chamber in the vessel and back to the chamber again for 1 h. The parameters used in the procedure were $E = 2.7$ kV/cm, with current approximately 2 A, duration of pulse 100 μs at 10 Hz of repetition frequency, where E was estimated as U/d, where U denotes the applied voltage and d denotes the electrode distance ($d = 15$ mm). Complete mixing was ensured by which agglomeration of cells was prevented. Each cell in the broth was retained in the treatment chamber for 2.1 s; i.e., exposed to a total of 21 pulses. After the procedure, the broth was left in a refrigerator for one week for the cells to sediment. Oil was skimmed off the surface into a 5 mL pre-weighted vessel and dried for 3 days in a dehydrator at 30 °C to avoid boiling. Finally, the vessel was cooled and reweighed. The calculation of oil yield was made by the mass difference between the empty, dried and cooled vessel with oil and the initial concentration of algal biomass in 2 L, which was 0.14 g. Experiments were performed on two different days.

3. Results

For the purpose of this study we developed a multi-switch square wave pulse generator, which was successfully used as a laboratory scale continuous flow system. We were able to generate a pulse amplitude as high as 5000 V, currents up to 80 A (Fig. 3), with rise time (220 ns), fall time (300 ns) and reliable continuous operation (up to 4 h and 12 h with a few minutes break between operations). We tested the system on two different flow chambers using different media and different cells, i.e., bacterial cells and microalgae.

3.1. Inactivation of bacterial cells

In our study, we observed the influence of electric pulses on bacterial viability using a developed square wave pulse generator. Bacterial cells were pumped through a laboratory scale continuous flow system and

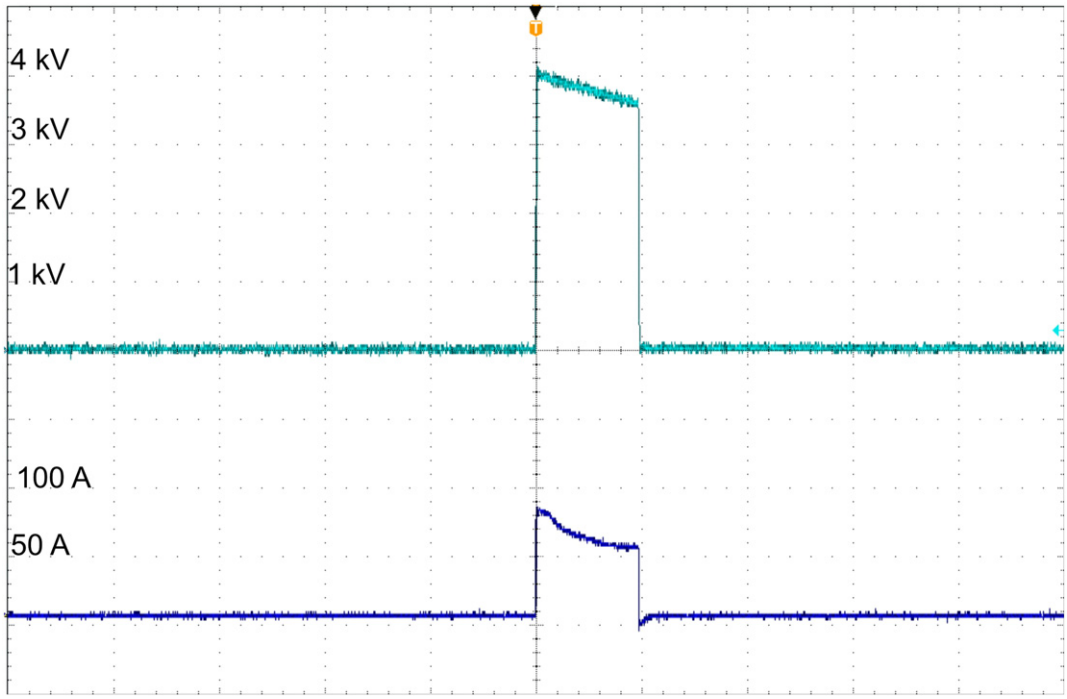

Fig. 3. Representative picture of high voltage pulse generated on a 50 Ω resistor and measured by oscilloscope (DLM 2024, Yokogawa, Japan) using a high-voltage probe (P6015A, Tektronix, USA) and current probe (Tektronix TCP0150). The figure shows the power capability of the square wave pulse generator (328 kW at the start of pulse, 216 kW at the end of pulse) but experiments were made at significantly lower power (*E. coli* max. 60 kW and algae max. 80 kW) and there was no noticeable drop in either voltage or current.

applied to one, four or eight 1 ms pulses of 12 kV/cm with a pulse generator operating at 10 Hz pulse repetition frequency. In Fig. 4, the inactivation level of *E. coli* cells suspended in H_2O to a 10^{-2} dilution and treated with electrical pulses at room temperature, and is reported as a function of the number of pulses. The results show that the inactivation level increased with an increasing number of pulses. By increasing the pulse number from one to eight at a fixed electric field strength and pulse duration (12 kV/cm and 1 ms, respectively), we achieved an almost 6-fold reduction. The treatment energy was 9.6 kJ/L, 38.4 kJ/L or 76.8 kJ/L when the bacterial cells were exposed to one, four or eight 1 ms pulses, respectively.

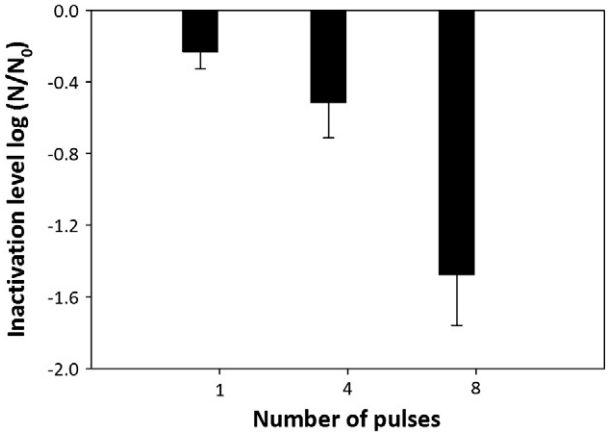

Fig. 4. The inactivation level of *E. coli* as a function of different numbers of 1 ms pulses at 12 kV/cm is shown. Bacterial cells (approximately 10^9 cells/mL) were placed in a laboratory scale continuous flow system. Pulses were applied at room temperature (22 °C). Values represent means ± standard deviation.

3.2. Microalgae treatment

Extraction with organic solvent is so far the most effective method for lipid extraction from microalgae. The generalization has therefore been made by which extraction with organic solvent (0.43 g lipid/g dry biomass) represents 100% yield of lipids from algal biomass. After applying 21 × 100 μs pulses using 4 kV in a co-field chamber with pulse generator operating at a repetition frequency of 10 Hz, we gained 22% lipids of cell dry mass, i.e., 51% when normalized to the extraction yield using organic solvent. The two tests were performed using algal cells from the same broth. In additional experiments, in which the treatment time was varied to 0.5, 1 and 2 h and the broth was thus exposed to 10, 20 or 40 pulses, 23%, 31% and 32%, respectively, lipids of cell dry mass were obtained normalized to the extraction yield using organic solvent. Energy is standardized per liter of the broth; 14.4 kJ/L of energy was therefore applied to the broth in 1 h, 7.2 kJ/L in 0.5 h and 28.8 kJ/L in 2 h.

4. Discussion

We developed and tested a square wave generator that enables continuous PEF treatment and is connected to a fluid-handling system and flow chamber (Figs. 1 and 2) with cross-field (*E. coli* treatment) or co-field electrodes (microalgae treatment) [67,68] since with large scale PEF treatment, flow processes are needed in order to treat large volumes of liquids. A square wave generator with a continuous flow system was tested for *E. coli* inactivation and microalgae oil extraction.

In electroporators designed for laboratory use, one of the main goals is to provide a wide range of pulse durations, frequencies and pulse amplitudes, as well as the required power to conduct experiments without the need to modify all media. The problem of generating pulses is adequately solved by serial stacked semiconductors controlled by optical fiber. This solution was shown to be reliable and affordable; moreover, it is possible to generate pulses in a wide range from 10 μs to 10 ms, at

voltages up to 5 kV and currents up to 100 A. The disadvantage, however, is too small an energy storage capacitor (100 μF), which is also the reason for a decline in the amplitude of voltage and current at the longest pulse width. Since the currents in our experiments did not exceed 10 A, this drawback was not relevant. The more important aspect is that we achieved fairly short rise (200 ns/4000 V) and fall (300 ns/4000 V) times. The average power (5 kV, conductivity 10 mS, 1 ms pulse duration) is 250 W for a frequency of 1 Hz and 2500 W for 10 Hz, which shows the limitation of our electroporator. The great advantages of our pulse generator are light weight (8 kg) and small dimensions (W/H/D) 32 × 27 × 20 cm, making the generator easy to transport.

The electroporation flow chamber is (in addition to the pulse generator) one of the key factors for achieving an effective process [51]. In our case, testing the flow chamber was more complicated because of the need to monitor the flow of fluid through the chamber, to monitor the temperature of the liquid and to monitor the high voltage pulse generator. We used two different chambers for testing: cross-field (for bacterial cells) and co-field (for microalgae) (Fig. 2). In laboratory experiments, various aims need to be achieved (microbial inactivation, extraction of valuable components from different cells — bacteria, yeast, and microalgae) for which different sizes and volumes need to be treated. It is therefore important to test the pulse generator on different loads; i.e., different chambers and different cell suspensions. When using a cross-field chamber, we had minor problems with leakage but, due to the substantial length of the electrodes and the low flow, this chamber allows better defined treatment of a liquid because of the homogenous electric field distribution within such a chamber as opposed to a co-linear chamber [74]. When using flow chambers, the flow rate and the geometry of the chamber must be taken into account in order to ensure that the cells in the liquid are exposed to the required number of pulses and to an appropriate electric field while passing through the chamber. In the *E. coli* inactivation experiments, therefore, the pulse frequency and flow rate in the continuous flow system were adjusted so that every bacterial cell in principle received a defined number of pulses. Our results indicate that inactivation of *E. coli* in a continuous flow system increased with the applied pulse number. It has been suggested, namely, that with aqueous pores created within the lipid membranes, membrane fragmentation also follows [75], possibly leading to cell death. Our results are in agreement with other studies [8,10,19,69,70] and comparable to the inactivation level of *E. coli* in our batch system (data not shown). It has been shown that increasing the pulse number or treatment time also increases the inactivation level of bacterial cells. In our study, we showed that when the pulse number was increased from 1 to 8, the inactivation level of *E. coli* rose approximately 6-fold.

Our pulse generator was also used in a continuous flow system in order to extract lipids from microalgae. Twenty-two percent of lipids of cell dry mass were obtained with 21 × 100 μs pulses using a 2.7 kV/cm voltage to distance applied in a co-field chamber pulse generator operating at 10 Hz without any pre-treatment of algae broth, compared with 43% lipids yield using organic solvent. The influence of exposure time of the algal broth to PEF on the yield of extracted lipids was also tested, with a variation of 0.5 h, 1 h and 2 h operating time. Lipid yields, however, were smaller than in the first experiment because algae had not been grown in "nitrogen starvation" conditions. Algae accumulate lipids only when the concentration of nitrate and ammonia ions is low. Otherwise the microalgae use the source of nitrogen to create lipids for cell membrane construction. When grown in a nitrogen rich environment, algae incorporate the produced lipids into their cell structures rather than accumulating them [36,40].

We must emphasize, however, that the quantification of extracted lipids via electroporation of microalgae is quite a challenge. Firstly, the analysis has to be performed immediately after the poration. Under certain electroporation parameters, as we have shown in later experiments, porated microalgae can reuse the extracted lipids and raw materials from the broth not just to survive but to gain more lipids in the cell.

The efficiency of lipid extraction via electroporation can be specified by two methods. The first is statistical and involves double electroporation. Cells rich with lipids are first porated in order to insert a type of colorant. This chemical reacts with lipids in the cell and creates luminance, which is quantified by photometric measurement. After the extraction, the process of lipid coloring in the cells is repeated. The difference between luminance before and after the extraction represents the efficiency of the process. Coloring cannot, however, be done on a whole sample. It requires a monolayer of algae cells. Statistical calculation shows the amount of extracted lipids in the whole broth. Sampling of cells may not be uniform; statistical deviation may consequently be high. By the second method, the lipid quantity in an algae cell can be determined by mass spectrometry.

Quantification of extracted lipids on a laboratory scale is a complex procedure. On an industrial scale, a huge amount of algae (for example 500 kg, which would give around 100 kg of lipids for the experiment) would be electroporated. The suspension would be kept in a sedimentation tank, in which the algae broth would separate due to gravity. Hydrophobic lipids would coagulate and float on the surface, where the layer could easily be skimmed off. Losses would be minor. Qualitative analysis could be performed using high pressure liquid chromatography or gas chromatography. It is crucial with these two methods that the sample contains no residual water.

Quantitative separation of lipids and water based algae broth on a laboratory scale is more complicated. The major problem is quantitative separation of the very thin lipid layer from the algae broth. In 60 L of algal broth with an algae concentration of 1.3 g/L and lipid extraction yield of 22%, 16 g of lipids float on the surface of the 60 L tank. Extraction of lipids with organic solvent may lead to subsequent losses. Our results on lipid extraction from microalgae therefore need to be considered only preliminary.

5. Conclusions

Our aim was to develop a pulse generator that enables PEF flow treatment and allows a pulse duration in a range from 10 μs to 10 ms and voltages up to 5 kV, with a maximum current of 80 A. Two different flow-through electrode chambers were tested for two different applications: inactivation of *E. coli* bacteria and extraction of oil from microalgae.

The inactivation levels of *E. coli* cells by PEF in a continuous flow system were determined and it was found that increasing the number of pulses also increases the inactivation level, as has already been shown in the literature.

Extraction of lipids from microalgae without expensive algal broth pre-treatment procedures, such as separation of phases and drying, promises cheaper production of biodiesel from microalgae. Usage of organic solvents represents potential environment contamination with poisonous chemicals and does not enable algal re-growth after lipid extraction. PEF is a promising method enabling clean, cheap, quick and simple extraction of substances from microalgae cells not only for biodiesel production but also for potential highly valuable products such as pharmaceuticals and dietary products.

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