Electrical Design of Electroporation Reactors

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***Abstract* - Electroporation, i.e. the treatment of biological cells by an electric field, is currently being introduced by industry in order to improve their production processes for food. Due to the variety of applications different electroporation reactors have been designed, most of them tailored for a specific application. Based on the design of some selected devices the paper describes the steps of the electrical design of an electroporation reactor. It starts with experiments in small scale in order to find out the required electric field strength and pulse shape. As a constraint due to requirements for the material and transport some devices have an inhomogeneous field distribution. But more advantageous is a substantially homogeneous field which usually is achieved either by a collinear design or an arrangement of parallel plate electrodes. In the paper both designs are compared to each other. For both geometries a method for easy scaling is presented enabling a fast adaptation of an existing design to the required size and throughput.**

***Index Terms* – Electroporation reactor, PEF treatment, Pulsed Power.**

I. Introduction

Electroporation is an emerging technology for opening plant cells in order to extract valuable substances. By applying an electric field to the biological tissue the cell membranes are charged and, subsequently, pores are formed in the membranes. If enough energy has been applied the membranes stay permeable and substances can be extracted.

As charging the cell membranes and pore formation is a fast process taking place within 100 ns to several microseconds a pulsed electric field is applied in order not to waste energy after cell opening, [1]. For the pulse application the tissue is placed between electrodes and the electric contact is established by means of a conducting fluid like water or juice. When applying a voltage pulse according to the conductivity of the material a current flow is involved.

For a continuous treatment of a product stream the electrodes are mounted inside treatment chambers and the product is moved through the chamber. But conductivity, required electric field and energy for complete opening, conveying properties, and required flow rates of products differ quite much. Hence, the treatment chamber has to be adapted to the needs of the application. For the design of the pulse circuit the treatment chamber can be considered in most cases as a ohmic resistor.

The circuit for pulse generation is chosen according to the required pulse shape and pulse width. For pulses in the microsecond range often RLC-circuits are used. A Marx generator may serve as a voltage multiplier to obtain easily a higher voltage. Fig. 1 shows an electroporation reactor for mash mounted above a Marx generator in a shielding housing. For shorter pulses generators based on transmission lines are used. They deliver rectangular pulses into a matched load. If the pulses are generated by a RLC-circuit the resistance of the treatment chamber influences the pulse shape. In case of a pulse application by an electrically long transmission line, an impedance match is crucial to omit reflections.

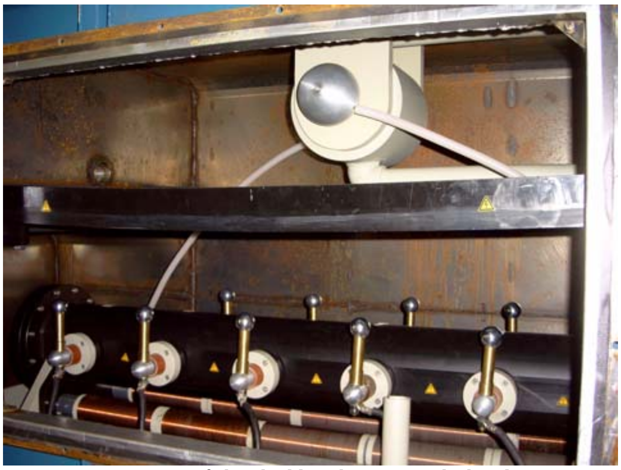

Fig. 1. Interior view of the shielding housing with the electroporation reactor and the Marx generator.

So far, different designs of treatment chambers have been developed and described in literature, [1,2,3,4]. The paper compares a choice of them from the electrical point of view.

II. Electric Properties of an Electroporation Reactor

A. *Biological Tissue in a Pulsed Electric Field*

1. *Charging the Cell Membranes*

Charging the cell membranes involves an exponential decay of the charging current when applying a rectangular pulse. In order to determine the charging constant for the tissue, experiments in a laboratory-scale electroporation device have been performed, [5]. As an example Fig. 2 shows the voltage across the electrodes and the current through the treatment chamber for a 1mm thin slice of sugar beet tissue of 1 cm diameter for the applied electric field strengths of 1 kV/cm, 2 kV/cm, and 3 kV/cm. The initial exponential decay of the current is superimposed by a subsequent increase due to the pore formation. The amount and speed of increase depend on the applied electric field.

To form pores in the cell membrane suitable for the extraction of substances a voltage in the order of 0.5 V across the membrane is needed, [6]. The required external field depends on the size of the cells. Larger cells bridge a larger volume collecting more voltage drop to charge the membrane or, in other words, less membranes are switched in series in a given volume of tissue. Hence, they require less external field strength.

2. *Repetitive Pulse Application*

Not all cells of a tissue are opened by the first pulse. Fig. 3 shows the measured current during 15 pulses of a pulse train. With increasing number of applied pulses the current shape approaches the rectangular shape of the applied voltage. The cell membranes of raw tissue have a comparable high specific resistance of 3000 Ohm cm², [7]. Current paths around them reduce the resistance of the tissue significantly. The pores formed in the membrane cause a further decrease of the resistance until the specific resistance of the cytoplasm is nearly reached.

In order to study the pore formation more detailed, the impedance of the sample has been measured between the pulses. Measurements at frequencies of 500 Hz and 5 MHz enable to distinguish between the resistance of the cytoplasm and contributions of the cell membranes. At higher frequencies the low impedance of the membranes' capacitances electrically bridges the membrane resistance. At low frequencies the resistance of the cell membranes increases the resistance of the sample. From these two measurements the constant resistance of the cytoplasm (R_s) and a resistance influenced by pore formation in the membranes and extraction of the cytoplasm (R_p) can be calculated, [8]. In addition the phase angle between applied voltage and current at f = 50 kHz, a frequency with nearly maximum phase shift for sugar beet tissue, has been determined. This phase shift depends on the degree of disintegration of the tissue.

The repetition rate has an influence on the electroporation efficiency. Fig. 4 and 5 show the decrease of the resistance R_p and the phase shift when applying 15 pulses at a pulse rate of 30 s and 600 s. The times of the pulse application are marked by dots along the x-axis. When applying the pulses at lower repetition rate each pulse triggers a decrease of resistance R_p and phase angle slowing down with the time. The continued change of R_p and the phase angle after the pulse application might be due to a continuing disintegration and extraction of cytoplasm between the pulses. If pulses are applied at higher repetition rate, they cause as well a decrease in resistance, but obviously less efficiently.

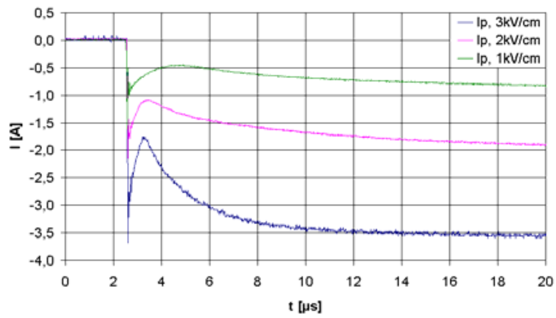

Fig. 2. Application of one rectangular pulse (t_p=20µs) to thin samples of sugar beet tissue at E = 1 kV/cm, 2 kV/cm, and 3 kV/cm.

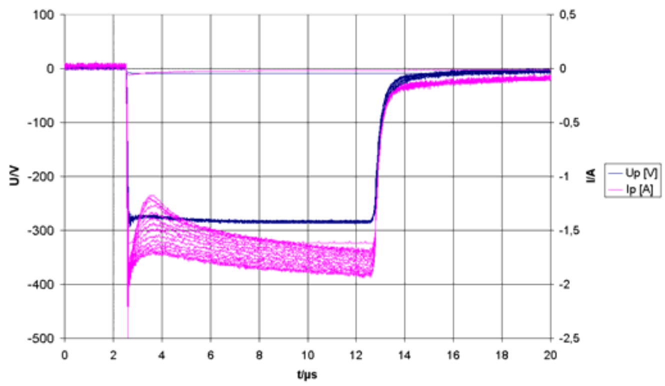

Fig. 3. Application of 15 rectangular pulses (t_p = 10µs) to a thin sample of sugar beet tissue at E = 2.8 kV/cm, and T = 20°C.

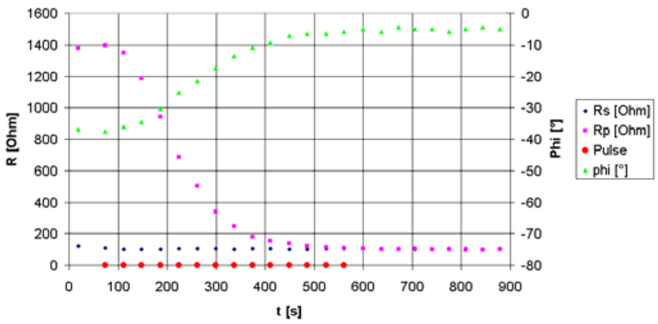

Fig. 4. Influence of the repetition rate: Application of 15 pulses at E = 2.8 kV/cm, t_p = 10µs, T= 20°C, and 30 s repetition rate to a thin sample of sugar beet tissue.

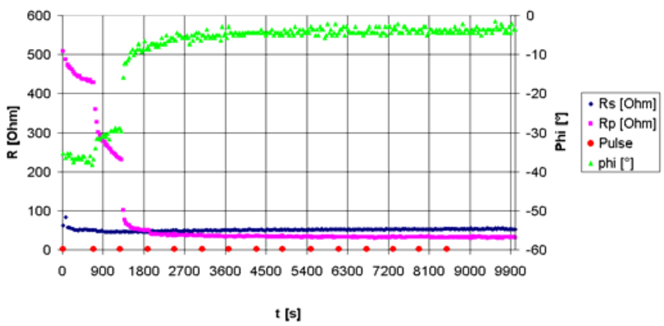

Fig. 5. Influence of the repetition rate: Application of 15 pulses at E = 2.8 kV/cm, t_p = 10µs, T= 20°C, and 600 s repetition rate to a thin sample of sugar beet tissue.

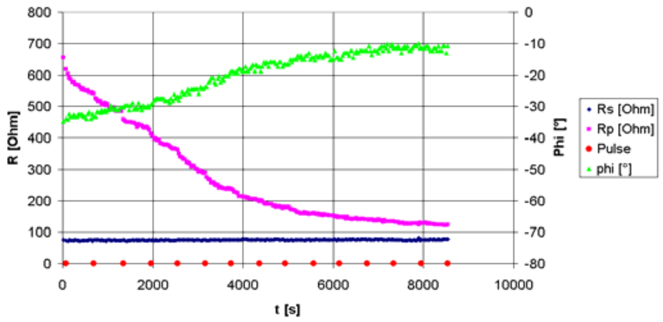

Fig. 6. Influence of the pulse width and applied external electric field: Application of 15 pulses at E = 1 kV/cm, t_p = 1.5µs, T= 20°C, and 600 s repetition pulse rate to a thin sample of sugar beet tissue.

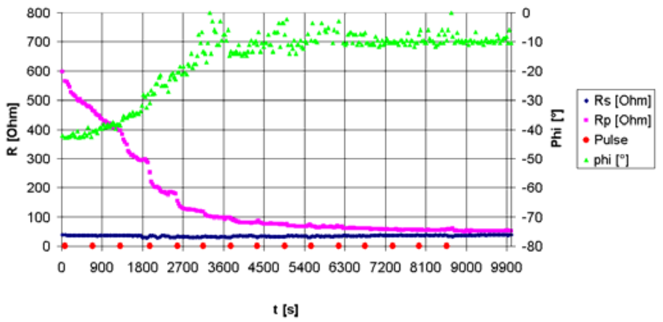

Fig. 7. Influence of the pulse width and applied external electric field: Application of 15 pulses at E = 1 kV/cm, t_p = 5µs, T= 20°C, and 600 s repetition pulse rate to a thin sample of sugar beet tissue.

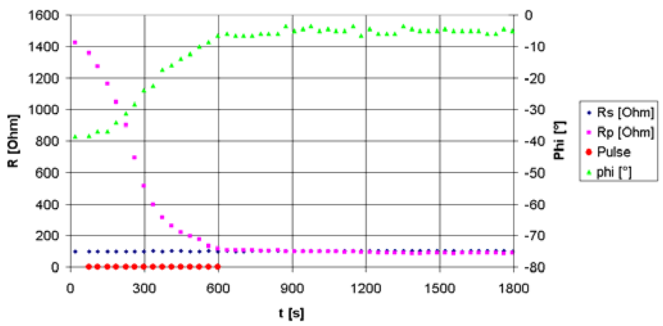

Fig. 8. Influence of the pulse width and applied external electric field: Application of 15 pulses at E = 2 kV/cm, t_p = 1.5µs, T= 20°C, and 30 s repetition pulse rate to a thin sample of sugar beet tissue.

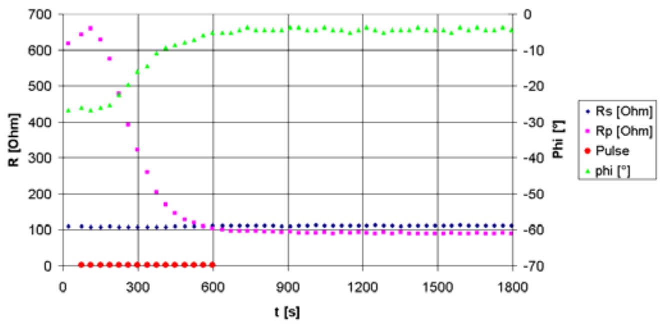

Fig. 9. Influence of the pulse width and applied external electric field: Application of 15 pulses at E = 2 kV/cm, t_p = 5µs, T= 20°C, and 30 s repetition pulse rate to a thin sample of sugar beet tissue.

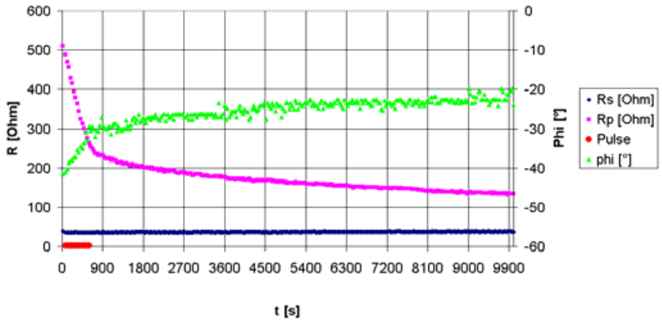

Fig. 10. Influence of the temperature: Application of 15 pulses at E = 1 kV/cm, t_p = 10µs, T= 10°C, and 30 s repetition rate to a thin sample of sugar beet tissue.

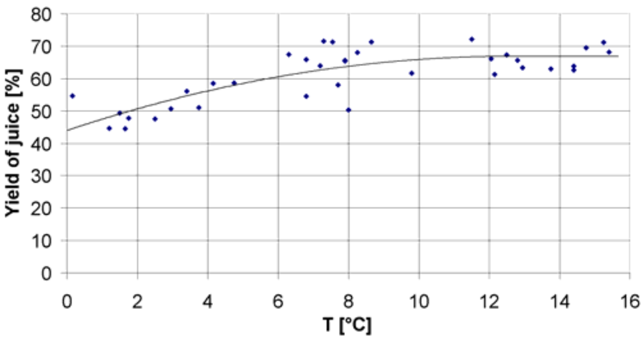

Fig. 11. Influence of the temperature: Yield of juice in percentage of the total sample weight after electroporation (30 aperiodically damped pulses, $\hat{E}$ = 3 kV/cm, t_h=1.66 µs), slicing, and subsequent pressing.

These effects might be explained by a different sensitivity of the cells of the tissue to the applied pulses. The local field distribution is governed by the alignment of the cells. The size of the cell influences the charging process of its membranes. Sensitive cells are opened first, and the cytoplasm is extracted locally. Due to the weakened structure of the tissue during subsequent pulses more robust cells are pulsed with higher local field strength. Hence, the tissue is opened stepwise.

For a large throughput in an industrial application a fast repetition rate of several Hertz is required. Therefore, a resting time before the further processing or a repeated treatment with some resting time between the pulse applications might be considered.

3. *Pulse Width*

The pulse width needs to be long enough to charge the cell membranes at the applied external electric field to the required voltage for pore formation. So at higher applied field the pulses may be shorter. In Fig. 6 and 7 at low electric field strength of 1 kV/cm the pulse width has been varied. At a pulse width of 1.5 µs, which is shorter than the time until a measurable increase of the current due to pore formation according to Fig. 2 there is only a slow decrease of the resistance R_p. For longer pulses with a length of 5 µs according to Fig. 7 until the 3rd pulse the decrease of R_p is similar, but then a strong decrease with each pulse has been observed. Now the tissue seems to be disintegrated to such an extend, that the local electric field at some cells is large enough to open the membranes such, that the influence on R_p is clearly visible. At a higher external field of 2 kV/cm a considerable disintegration of the tissue takes place independent of the pulse width between 1.5 µs and 5 µs (Fig. 8 and 9).

4. *Temperature Dependence*

Apart from the size of the cells the electroporation efficiency depends as well on the temperature. Fig. 10 shows the disintegration of slices of sugar beets at 10°C due to the application of 15 rectangular pulses of 1 kV/cm and 10 µs pulse width. At that low temperature the applied energy is not sufficient for a complete opening of the cells. Additionally, Fig. 11 shows the temperature dependence of the extracted juice from samples of sugar beets during pressing after treatment with 30 pulses of 3 kV/cm at 1.66 µs pulse width, [5]. For this experiment aperiodically damped pulses have been applied.

Due to the complex mechanisms taking place during electroporation, which are not yet fully understood, for a new design of an electroporation device experiments with the material to be treated are required. Table 1 lists some operation parameters of devices which have been designed so far.

TABLE I

DESIGN DATA OF ELECTROPORATION DEVICES

	Device 1	Device 2	Device 3
Material	Sugar beets	energy crop (grass, maize, rye)	grape mash
Ê [kV/cm]	2 – 5	5	35 - 40
t_h [µs]	1.2 - 1.5	5 - 8	1.2 -1.5
f_{rep} [Hz]	20	5	20
Reference	[1,5]	[9]	[3]

B. *Electric Field in a Conductive Medium*

In a conductive medium the electric field consists of a capacitive and a conductive component. The high-frequency range is governed by the capacitive field distribution, while for lower frequencies the conductivity determines the field distribution. Whether for a pulsed application either of these simplifications can be applied depends on the relaxation time $\tau = \varepsilon_0\varepsilon_r / \kappa$. Many media to be electroporated have a conductivity κ between 1 mS/cm and 4 mS/cm. With the relative permittivity ε_r for water of 81 the relaxation time is in the order of 7.2 ns to 1.8 ns. So for pulses in the 100 ns- to microsecond range the capacitive components of the electric field can be neglected in order to facilitate field calculations.

In order to establish a homogeneous field distribution across the cross section of the electroporation reactor, a homogeneous current distribution is important.

The distribution of alternating or pulsed current inside a conductor is governed by eddy currents. For a cylindrical conductor the depth of current penetration d can be calculated according to (1) (skin effect), [10].

$$d = \sqrt{\frac{1}{\pi f \ \kappa \ \mu_0 \mu_r}} \,. \qquad (1)$$

It is determined by the frequency f of the applied current, the conductivity κ, and the magnetic permeability $\mu_0\mu_r$ of the processed material (μ_r=1). Whether for a pulsed current a homogeneous current distribution can be achieved depends on the highest considerable frequency component and the dimensions of the treatment chamber. As an example Fig. 12 shows shape and spectral density of a current pulse through the electroporation reactor of the device for grape mash (device 3 according to Table 1), [3]. The penetration depth for the current can be calculated to 1.3 m with a pulse length of t_h=1.2 µs resulting in an 3dB-limit at 500 kHz and a conductivity of 3 mS/cm. As this is much more than the actual dimensions of the treatment chamber of less than 10 cm, a homogeneous current distribution across the cross section can be assumed. Even at 10 MHz the penetration depth would be 29 cm.

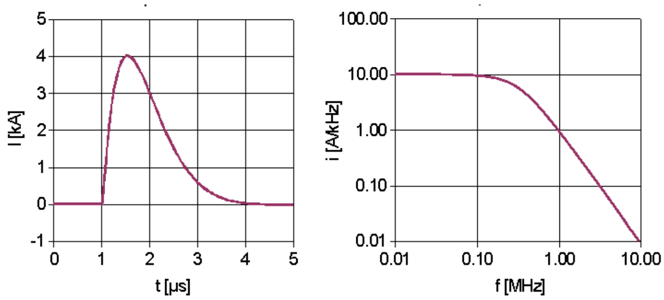

Fig. 12. Shape and spectral density of a current pulse through the electroporation reactor of an electroporation device for grape mash (PSPICE circuit simulation).

The numeric field calculations shown in the following paragraph have been performed using the charge simulation method, [11]. It can be used for field calculations in a conductive medium when considering the analogies between electric and conductive field according to Table 2. For the simulation of the conductive field the charges representing the electrode borders during electrostatic field calculation are interpreted as current sources. Then the electric flux density D corresponds to the current density J and the charge of an electrode Q corresponds to the current injected by an electrode I. The resistance R of an electrode system can be calculated based on the injected current and the voltage V between the electrodes, much like the calculation of the capacitance C in the electrostatic case.

TABLE 2

ANALOGIES BETWEEN ELECTROSTATIC AND CONDUCTIVE FIELD.

Electrostatic field	Conductive field
$\vec{D} = \varepsilon \cdot \vec{E}$	$\vec{J} = \kappa \cdot \vec{E}$
$\iint \vec{D}\, d\vec{A} = Q$	$\iint \vec{J}\, d\vec{A} = I$
$\vec{E} = -grad\ \varphi$	
$div\ grad\ \varphi = 0$	
$C = \frac{Q}{V}$	$\frac{1}{R} = \frac{I}{V}$

So far for the modeling a medium of homogeneous conductivity has been assumed. The plant material consists of a suspension of tissue, which is in microscopic scale inhomogeneous. But for the design of the electroporation reactor the macroscopic behavior is of importance. According to the step response (Fig. 3) the increase of the current from pulse to pulse can be considered macroscopically as a variable conductivity of the material. In the beginning of the charging process and as well after complete opening of the cells the resistance of the electroporation reactor is governed by the conductivity of the cytoplasm. Hence, this conductivity can be used for the design of electroporation reactor and pulse circuit. For raw plant material the resistance is increased.

When the required pulse shape and electric field strength to be applied to a medium is known from experiments, an electroporation reactor can be designed based on field calculations or scaling of existing designs:

III. Design of an Electroporation Reactor

A. Field Distribution

1. Homogeneous Field

For a homogeneous treatment of the material independent of its path through the treatment zone a substantially homogeneous field is crucial. A homogeneous field might be established in a plate electrode system in a closed chamber with the insulating walls limiting the field region. Inlet and outlet for the product stream involve inhomogeneities of the field. Two main designs of treatment chambers with a homogeneous field differ concerning the field orientation with respect to the flow direction: The collinear design has tubular shaped electrodes connected by an insulating tube in between, Fig. 13, right. Usually it is arranged in a three electrode system with the outer electrodes on ground potential and the inner electrode on high-voltage potential forming two treatment areas. Hence, the electric field is oriented in or against flow direction. Electrically both treatment areas are switched in parallel. The parallel-plate design, Fig. 13, left, consists mainly of two parallel plate electrodes with the material passing the gap between them. Hence, the electric field orientation is in normal direction to the flow direction. For a safe operation of the treatment chamber and also for a good efficiency it is important, that no current flows out of the electroporation area in direction to the inlet or outlet of the electroporation device. When using the collinear design inlet and outlet electrodes may be connected safely to ground potential. If a plate-electrode system is fed by a voltage source unsymmetrical to ground, current may flow out of the treatment area. This can be omitted by feeding the electrode system symmetrically to ground potential. If the plant material fills the electrode gap homogeneously the center plane between the electrodes is virtually on ground potential. But for safety reasons for the case of an asymmetry of supply voltage or material distribution additional ground electrodes in some distance from the treatment zone should be added. The ground symmetric operation has the advantage, that the voltage to ground is only half of the voltage between the electrodes, hence, enabling a more compact design of the device than with the collinear design due to a reduced insulation distance to ground.

2. Varying Field Direction

For non-spherically shaped cells the field orientation relative to the cell is of concern. Some designs take this fact into account by rotating the field orientation in order to apply the field to the cells along their longitudinal axis, [1]. The electroporation reactor of the electroporation device KEA-MOBIL consists of an insulating cylindrical tube with round shaped nearly flat electrodes with a diameter of 3.5 cm mounted into the inner surface of the tube. Three pairs of electrodes are placed along its axis, the electrodes of each pair opposite to each other, but with their axis rotated by 45° with respect to the next electrode pair in flow direction, Fig. 14. Hence, when moving through the reactor the field changes its orientation. But due to the small size of the electrodes the field distribution is inhomogeneous.

3. Electrode Profile

For the treatment of small cells a higher electric field strength is required. In order to omit corona discharges initiating a flash-over inside the treatment chamber the electrode profile can be adapted accordingly. In order to enable easy manufacturing, the electrode shape has been approximated by radii. For the plate electrode system shown

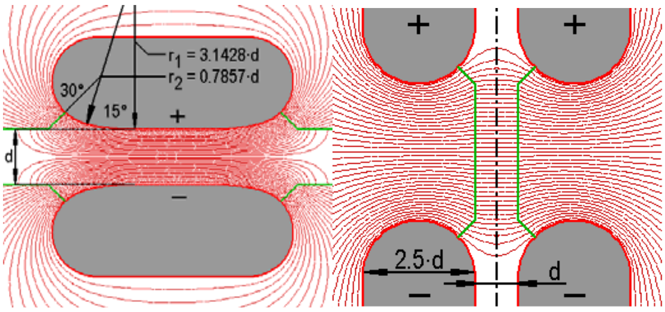

Fig. 13. Examples for a plate-electrode design (left) and a collinear design (right) showing equipotential lines and construction details.

Fig. 14. Electroporation reactor with rotated field orientation along its axis and round shaped electrodes (lower left corner).

in Fig. 13 the radii of the electrodes have been optimized by iterative field calculations in such a way, that the field at the borders of the electrodes does not increase significantly. Known electrode profiles according to Borda or Rogowski are designed for the use in a homogeneous medium. As the diameter of the channel has to be kept nearly constant in order to prevent the treated material from blocking, insulating material at both sides of the electrode is required. But it causes an additional enhancement of the conductive electric field which has to be compensated by the electrode shape. The surfaces of insulator and electrode are arranged in an angle of 90° in the high-field region near the electrode's surface. Hence, a field enhancement in the insulator near this triple point connecting three media can be omitted. If only a lower field strength is required this angle might be changed. So a flatter channel surface can be obtained. But care has to be taken on the field enhancement in the insulator as material of lower conductivity, if one field line crosses insulator and the material for treatment. The cross section of the channel between the electrodes is rectangular. The electric field inside the insulating walls is resistively controlled by the conducting medium in the vicinity. The borders of the electrodes are embedded into the insulator completely preventing a flash-over in air outside the electroporation reactor. In order to obtain a small surface to volume ratio for a good transport of the material a square cross section is preferable.

For the cylindrical collinear design shown in Fig. 13 at the junction between the electrode and the insulator the insulator surface is in parallel to the direction of the electric field and a 45° angle has been chosen to keep the tube diameter constant. The electrode has a toroidal shape. The ratio of the diameters influences the field enhancement near the electrode. A tradeoff between size and field enhancement has to be made. It is essential not to exceed the field strength for the inception of surface discharges along the insulator's surface.

B. *Scaling the Design*

If a suitable electrode shape has been found, it can be scaled easily to the required size by multiplying x- and y-axis by the appropriate scaling factor. In addition, the region with a homogeneous field can be stretched according to the needs. So in order to keep the low-field regions near inlet and outlet small, the cross section should be as small as possible for transporting the material through the treatment area. Dependent on the field orientation scaling and stretching has a different influence on the electrical characteristics of the treatment chamber. Considering the homogeneous field area, in a collinear design the applied voltage for a given field strength scales with the length. According to (2),

$$R = \frac{1}{\kappa} \cdot \frac{l}{A}, \tag{2}$$

the resistance R increases in proportion to the length l of the homogeneous-field region and decreases for a larger area A. For the described plate electrode system with square cross section the applied voltage needed to establish a required field strength scales with the electrode distance and, hence, with the square root of the cross section area. According to (3),

$$R = \frac{1}{\kappa} \cdot \frac{d}{A} = \frac{1}{\kappa \cdot l} \quad with\ A = d \cdot l\ , \tag{3}$$

the resistance R scales inverse proportional to the length l of the reactor, but it is independent of the cross section. Hence, comparing two treatment chambers of the same volume and cross-section, but with different field orientation the collinear design has a higher resistance, but requires as well a higher voltage than the plate electrode system to establish the electric field. The electric power to establish the electric field in the homogeneous-field area in the conductive medium is for both designs the same. Hence, the plate electrode design is more suitable for compact designs, but requires a larger current for operation.

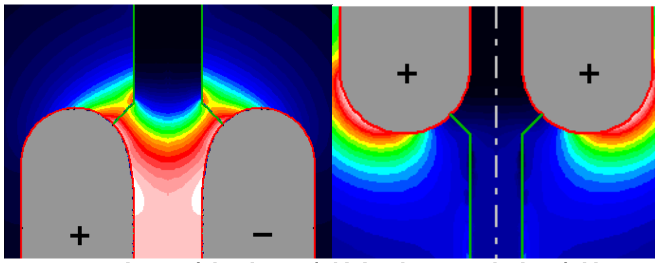

Fig. 15. Simulation of the electric field distribution in the low-field region near the inlet: left: plate-electrode design, right: collinear design.

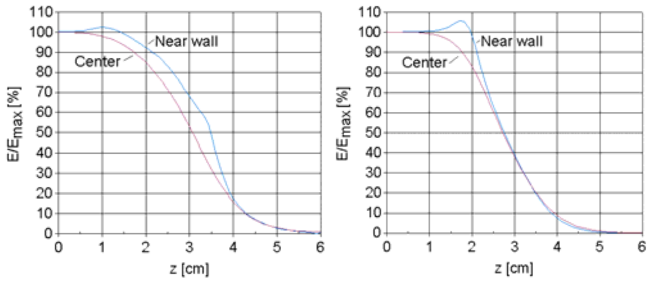

Fig. 16. Relative electric field strength in the low-field region near the inlet along paths in the center and near the wall of the electroporation reactor; left: plate-electrode design, right: collinear design (cross section A = 324 mm²).

C. *Losses due to Low-Field Regions*

If the electric field strength is not sufficient to achieve the membrane voltage required for a considerable pore formation only losses occur due to ohmic heating of the medium. The losses in the low-field regions near inlet and outlet depend on the actual shape of the electrodes. Small low-field regions reduce the losses. For two treatment chambers of same size the relative losses due to low-field regions can be compared. Fig. 15 shows a collinear and a plate-electrode design, both with the same cross-section. In their inlet- and outlet sections the electric field decreases according to the diagrams in Fig. 16. In both diagrams the electric field strength is related to the field strength E_{max} in the homogeneous-field region in the center and near the wall. The origin of the z-axis (z=0) has been set to the start of the inlet- or outlet section. The collinear design exhibits a slightly steeper decrease of the field strength resulting in a smaller low-field region. But taking into account that for a complete electroporation reactor either two treatment chambers or a long feeding tube is required, in total the low-field region is larger.

D. Scaling the Resistance

For an easy estimation of the resistance based on scaling without additional field calculations the treatment chamber can be divided into three sections: the middle section with the homogeneous field region and the inlet- and outlet-sections covering the regions with an inhomogeneos field. For the plate-electrode design the sections are separated by a cut in the direction of the electric field, for the collinear design normal to the field direction. So all three sections can be considered independent of each other as resistors, which are switched in parallel or series, respectively. When scaling the ratio of the sizes of electrode surfaces and volumes stays the same. Hence, the ratio of the resistances does not change. For an easy scaling a constant factor between the resistance of an inhomogeneous field region and the resistance of the homogeneous field region with a fixed length can be calculated. Instead of the resistance of the homogeneous field region as well a geometric parameter for scaling can be chosen, for example the diameter of the channel. But then the conductivity has to be considered additionally.

For the two designs shown in Fig. 13 the scaling factors have been calculated by means of numeric field calculation and are listed in Table 3. From the mechanical design of the electroporation reactor, which includes the calculation of the required tube cross section based on desired throughput and velocity, the electrode distance d_e or channel diameter d_c are known. With the conductivity κ, the desired repetition rate and number of applied pulses, the length of the treatment area can be calculated. This is either the length of the homogeneous field area l or, if a decrease of the electric field strength by 10 % in the center is acceptable, according to Fig. 16 2 · 1.7 cm can be added. The total resistance of the electroporation reactor then can be estimated according to Table 3. For an easy use the described calculations may be implemented into a computer program or spread sheet calculation.

IV. Conclusion

The electrical design of an electroporation reactor is based on experiments in order to find out the required pulse parameters for the desired processing material. Subsequently, the high-voltage design of the treatment chamber is done. For the parallel-plate and the collinear electrode arrangement a scaling process can simplify the design. So some common designs might be done without a deep knowledge in electric field calculation.

TABLE III

Scaling Factors for Two Electroporation Reactor Designs

	Plate-Electrode Design	Collinear Design
Resistance of middle section R_C	$R_C = \frac{1}{\kappa \cdot l}$	$R_C = \frac{l}{\kappa \cdot 0.25\pi \cdot {d_t}^2}$
Resistance of inlet- and outlet section R_{IO}	$R_{IO} = \frac{0.67}{\kappa \cdot d_e}$	$R_{IO} = \frac{0.32}{\kappa \cdot d_t}$
Total resistance R	$R = \frac{0.5 \cdot R_{IO} \cdot R_C}{0.5 \cdot R_{IO} + R_C}$	$R = 2R_{IO} + R_C$
Length of treatment area l_t	$l_t = l + 1.88 \cdot d_e$	$l_t = l + 1.7 \cdot d_t$

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