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Electroporation-based applications in biotechnology

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Electroporation is already an established technique in several areas of medicine, but many of its biotechnological applications have only started to emerge; we review here some of the most promising. We outline electroporation as a phenomenon and then proceed to applications, first outlining the best established – the use of reversible electroporation for heritable genetic modification of microorganisms (electrotransformation), and then explore recent advances in applying electroporation for inactivation of microorganisms, extraction of biomolecules, and fast drying of biomass. Although these applications often aim to upscale to the industrial and/or clinical level, we also outline some important chip-scale applications of electroporation. We conclude our review with a discussion of the main challenges and future perspectives.

The phenomenon of electroporation

Exposure of biological membranes to a sufficiently high electric field leads to a rapid and large increase of their electric conductivity and permeability. This effect – membrane electroporation – can be either reversible or irreversible, and was first reported for excitable cells in 1958 [1], for nonexcitable cells in 1967 [2], for planar lipid bilayers in 1979 [3], and for lipid vesicles in 1981 [4].

Molecular level

Both theoretical considerations [5] and molecular dynamics simulations [6] imply that electroporation is initiated by penetration of water molecules into the lipid bilayer of the membrane, causing reorientation of the adjacent lipids with their polar head groups toward these water molecules. The pores formed in the cell plasma membrane provide a pathway for transport of a wide range of molecules, including DNA, into and out of the cell. Electroporation is a physical phenomenon, and can as such occur in the lipid bilayer of the membranes of all prokaryotic and eukaryotic cells.

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Membrane level

Pore formation is governed by statistical thermodynamics [5,7], and it is therefore not strictly a threshold event in the sense that pores only form in electric fields that exceed a certain value. Nonetheless, electroporation-mediated transport across the membrane correlates strongly with the transmembrane voltage induced by the external electric field, which until the onset of electroporation is proportional to this field [8] and then decreases [9]. There are four general contiguous ranges of electric field strength (Figure 1), each characterized by typical properties of the pores formed and/or transport through them [10]. In the range of no detectable electroporation, the pores, even if formed, are too small and short-lived for measurable transport. In the range of reversible electroporation, pores provide a temporary pathway for transport, but after the electric pulse they gradually reseal, the transport ceases, and most cells retain their viability. In the range of nonthermal irreversible electroporation, most pores either do not reseal, or reseal too slowly to preserve cell viability; cells thus gradually disintegrate and release their contents, yet these contents are not thermally damaged. Finally, in the range of irreversible electroporation with thermal damage, electric current causes a temperature increase sufficient to cause thermal damage to the released molecules (protein denaturation above ~50 °C, DNA melting above ~70 °C).

The four ranges partly overlap because pore formation is stochastic and cells generally vary in size and/or orientation with respect to the field. The range boundaries depend on the cell type and are affected by the properties of the medium in which the cells are exposed – its electrical conductivity, osmolarity, and the solutes it contains [11,12]. As the exposure duration (i.e., electric pulse length) increases, the transitions between adjacent regions occur at lower fields (Figure 1A). The range of detectable poration, however, has an asymptotic lower bound – below a certain field strength, no transmembrane transport is detected no matter how long the applied pulses [13,14].

The electroporation-induced pores have not yet been visualized directly; with radii of at most several nanometers, they are too small for optical microscopes and too unstable – because the lipid bilayer is fluid – for the preprocessing required for electron microscopy of soft

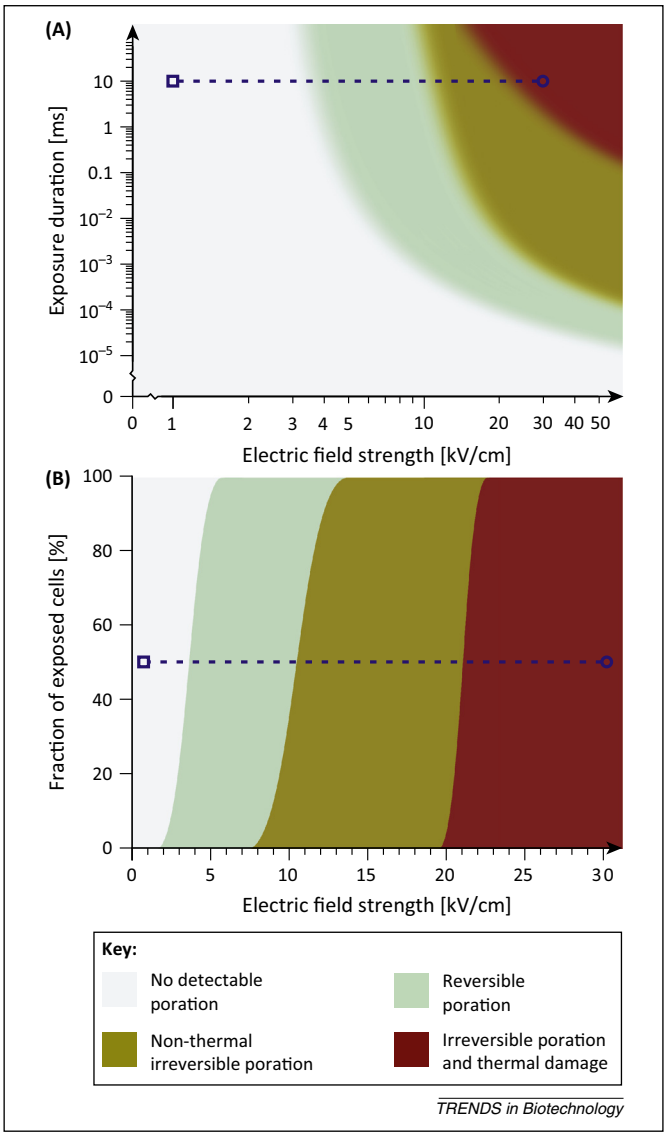

Figure 1. Membrane poration and thermal effects during exposure of cells to an electric field. The values are for typical bacteria in 0.25 M sucrose. **(A)** Ranges of no detectable poration, reversible poration, irreversible poration, and thermal damage as functions of field strength and duration. **(B)** Fractions of non-porated, reversibly porated, irreversibly porated, and thermally damaged cells as functions of field strength, for a 10 ms exposure time [i.e., the plot in panel (B) traces these fractions along the dashed line segment in panel (A)]. Note that the axes are logarithmic in (A) and linear in (B). Adapted, with permission from [10].

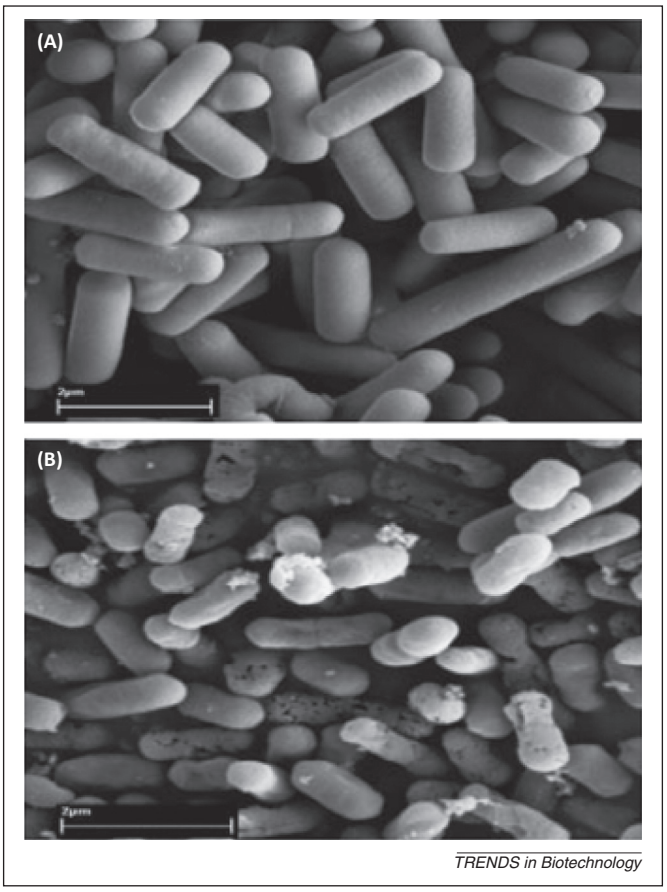

Figure 2. Electroporation of bacteria. Scanning electron micrographs show *Lactobacillus casei* bacteria before **(A)** and after **(B)** exposure to an electric field pulse of 7.5 kV/cm amplitude and 4 ms duration. Reprinted, with permission from [16]. Scale bar, 2 μm.

matter, and early reports of volcano-shaped pores tens of nanometers in size are now known to have been artifacts of such preprocessing [15]. By contrast, the pores in the cell wall, particularly in the peptidoglycan wall of Gram-positive bacteria, are much more stable and can be visualized under an electron microscope [16] (Figure 2).

Tissue level

Exposure to a sufficiently strong electric field also causes electroporation in multicellular tissues, enhancing transport into or out of their constitutive cells. Uniform electroporation is difficult to obtain in tissues because they generally consist of diversely shaped cells, various cell types (including vascularization), and cells connected by gap junctions, resulting in spatially varying and often anisotropic electrical properties. Thus, even if a tissue is exposed to a homogeneous external electric field, inside the tissue the field is distributed highly nonhomogeneously, and some cells are almost unavoidably electroporated more intensely than others [17,18].

To reduce field nonhomogeneity the electric field delivery to a tissue must be carefully designed; a numerical model of the tissue is built, taking into account its particular structure; the number, size, shape, and positioning of the electrodes are then iteratively optimized until sufficient field homogeneity is achieved inside the tissue or in a sub tissue of interest, for example, a tumor inside a larger tissue such as the liver [19].

Once the tissue cells are electroporated, the electric conductivity and dielectric permittivity of the tissue change, affecting the electric field distribution [20]. Particularly when more than one electroporating pulse is delivered, these dynamic changes must also be considered for optimal results. In such applications, numerical modeling is complemented by real-time measurements of tissue conductivity acquired with the electrodes that are also used for electroporation, allowing subsequent pulses to be adapted to the detected increase of conductivity reflecting the extent of electroporation [21].

While electroporation is already an established technique in several areas of medicine, many of its biotechnological applications have only started to emerge. There are four general types of such applications (Box 1), and we describe each of them in more detail.

Box 1. Applications of electroporation in biotechnology

Electroporation both allows exogenous molecules to be introduced into cells and endogenous molecules to be extracted from within the cells, resulting in four general areas of biotechnological exploitation (Figure I). In electrotransformation, exogenous DNA is introduced by means of reversible electroporation, the foreign genes are expressed in their new host cells and they are inherited upon cell division; this can turn the host microorganisms into 'factories' of biomolecules, adapt them to a new environment, or serve to study the role of individual genes. In electroporation-based inactivation, microorganisms are exposed to electric field pulses strong and long enough to inhibit their activity, including their division, growth, and synthesis of pathogenic substances. This method avoids contamination and is particularly promising in food preservation where radiation and chemicals must be avoided for the obvious reasons, while heating degrades both nutrients and taste, decreasing the value of food. In electroextraction, either microorganisms or multicellular tissues are electroporated to the extent required to release the biomolecules of interest; in some cases, it is also achievable with reversible electroporation and, in most cases, it is important to limit electroporation to levels that avoid rapid decomposition of the exposed cells and thus the formation of debris contaminating the extract. Finally, when used for facilitating water release from tissues, electroporation is useful in electroporative biomass drying, accelerating the drying process, allowing heating to be reduced or avoided, and often also reducing the energy requirements.

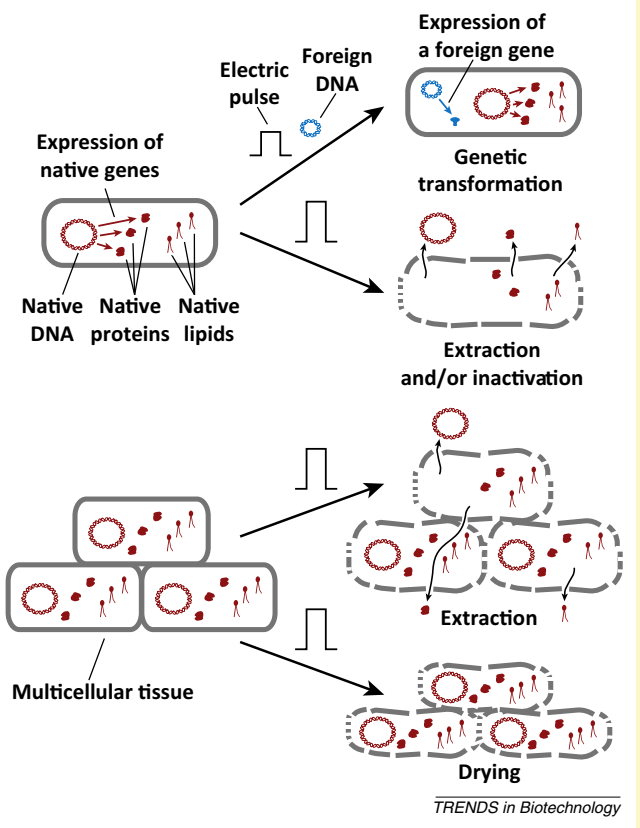

Figure I. Applications of electroporation in biotechnology.

Genetic transformation of microorganisms

Although some microorganisms can spontaneously transform – take up foreign (heterologous) genes, express and replicate them, and pass them on upon division – the efficiency is often low, and there is ample motivation for controlled artificial transformation. Many approaches have been attempted, ranging from chemical and mechanical to thermal, but since the mid-1980s transformation based on electroporation (electrotransformation) has prevailed because it is more efficient and is applicable to a broader range of bacteria [22].

In early 1990s electrotransformation was also shown to be effective in archaea, unicellular algae (microalgae), and unicellular fungi (yeasts), although with some limitations. Halophilic archaea cannot tolerate NaCl concentrations below 1 M, but in media of such salinity the electric field required for electroporation generates organism-damaging heating, hence electrotransformation may be unfeasible. In some other archaea, despite attempts at optimization, researchers were unable to detect transformants [23], and recent studies imply that some archaeal lipids are indeed highly resistant to the reorientation required for pore formation, and this could hinder electrotransformation [24,25]. In microalgae and yeasts, transformation efficiencies are generally lower than in bacteria and most archaea, but still sufficient for some applications.

To date, successful electrotransformation has been reported for bacteria from at least 13 of the 29 currently recognized taxonomic phyla, for archaea from at least two of their five phyla, for microalgae from at least three of their six phyla, and for yeasts from both their phyla (Table 1).

Applications of electrotransformation

Production of biomolecules. Most frequently, electrotransformation is used for synthesis of foreign substances, including antigens, cytokines, enzymes, hormones, and toxins, in host organisms ranging from bacteria to microalgae and yeasts. New transgenic 'factories' of biomolecules are emerging at a formidable rate; already in 1995, 4 years after first electrotransformation-mediated transgenic protein production in yeast, a review listed 22 such proteins produced in a single yeast species, and 15 in another [26].

Adaptation to diverse conditions. Some microorganisms perform useful functions, for example, probiotics in the intestine, or bioremediators of environmental pollutants, and transformation can adapt them to new antagonists or environments. Thus, the bacterium *Deinococcus radiodurans* is an efficient bioremediator of uranium, converting it from soluble hexavalent into the insoluble tetravalent state, thus preventing leakage from nuclear waste storages, but it cannot survive above 40 °C, which is often exceeded in high-density storages; electrotransfer of its genes encoding uranium chemistry into its thermophilic relative *Deinococcus geothermalis* produced a strain converting uranium at temperatures up to 55 °C [27].

Basic research. Transfer of a gene into a simpler organism can facilitate analysis of the properties and functions of the encoded protein, which may be obscured in the more-complex original organism. If the transgenic protein interacts with host genes or their expression, electrotransformation can also be used to study the genes and proteins of the host organism. For example, in the bacterium *Brachyspira hyodysenteriae* the flagella consist of two types of proteins encoded by two genes, and transformation that inhibited one gene resulted in thinner flagella (~20 instead of ~26 nm diameter) with reduced motility [28].

Table 1. A sample of successfully electrotransformed microorganisms

Phylum	Species
Archaea	
Crenarchaeota	*Metallosphera sedula, Sulfolobus acidocaldarius, Sulfolobus islandicus, Sulfolobus solfataricus*
Euryarchaeota	*Methanococcus voltae, Pyrococcus furiosus*
Bacteria	
Actinobacteria	*Brevibacterium lactofermentum, Corynebacterium diphtheriae, Mycobacterium smegmatis,...*
Bacteroidetes	*Bacteroides fragilis, Bacteroides uniformis, Prevotella ruminicola,...*
Chlamydiae	*Chlamydia psittaci, Chlamydia trachomatis*
Chlorobi	*Chlorobium vibrioforme*
Cyanobacteria	*Arthrospira platensis, Fremyella diplosiphon, Synechococcus elongatus*
Deinococcus-Thermus	*Deinococcus geothermalis, Thermus thermophilus*
Firmicutes	*Bacillus cereus, Clostridium perfringens, Enterococcus faecalis, Lactobacillus casei, Streptococcus pyogenes,...*
Fusobacteria	*Fusobacterium nucleatum*
Planctomycetes	*Planctomyces limnophilus*
Proteobacteria	*Campylobacter jejuni, Escherichia coli, Salmonella enterica, Sinorhizobium meliloti, Yersinia pestis,...*
Spirochaetes	*Borrelia burgdorferi, Serpulina hyodysenteriae*
Tenericutes	*Mycoplasma pneumoniae*
Thermotogae	*Thermotoga maritima*
Unicellular algae (microalgae)	
Chlorophyta	*Chlamydomonas reinhardtii, Chlorella ellipsoidea, Chlorella vulgaris, Dunaliella salina, Scenedesmus obliquus,...*
Heterokontophyta	*Nannochloropsis sp. W2J3B, Phaeodactylum tricornutum*
Rhodophyta	*Cyanidioschyzon merolae*
Unicellular fungi (yeasts)	
Ascomycota	*Candida maltosa, Ogataea polymorpha, Pichia pastoris, Saccharomyces cerevisiae, Schizosaccharomyces pombe,...*
Basidiomycota	*Cryptococcus neoformans, Pseudozyma antarctica, Pseudozyma flocculosa*

Parameters affecting electrotransformation efficiency

Field strengths for optimal transformation efficiency range from 1 to 20 kV/cm, and pulse durations from 1 to 30 ms; in general, optimal values of these two parameters are those resulting in the highest extent of electroporation without substantial loss of viability. For a given microorganism and DNA molecule, the optimal values of these two parameters must in general be determined empirically [22]. Other factors influencing electrotransformation efficiency are discussed below.

Organism envelopes and growth phase. Transformation efficiency decreases with increasing thickness and number of layers enveloping the DNA of the recipient [29]; thus, achievable efficiencies are highest for Gram-negative bacteria (10^7–10^{10} transformants per μg DNA), lower for Gram-positive bacteria and archaea owing to their thicker cell wall (10^5–10^7), and even lower for microalgae and yeasts that have a nuclear membrane (10^4–10^7) or for organisms that have an outer polysaccharide- or slime-capsule layer, such as some bacteria and archaea during particular growth phases ($\leq 10^4$). Bacteria and archaea are thus optimally transformed in their exponential growth phase [30], in which the capsular synthesis rate decreases [31].

Organism size. Because the transmembrane voltage induced by exposure to a given field strength is proportional to the size of the organism, the field required for electroporation and thus transformation is larger for smaller organisms; thus, optimal field strengths are generally higher for bacteria and archaea (5–20 kV/cm) than for microalgae and yeasts (1–12 kV/cm).

DNA. Transformation efficiency is highest for supercoiled circular double-stranded (ds) DNA, and increasingly lower for relaxed circular dsDNA, circular single-stranded (ss) DNA, linear dsDNA with homologous ends, and linear dsDNA with nonhomologous ends [32]. For DNA concentrations from pg/ml up to μg/ml, transformation efficiency is roughly constant, implying that within this range, and under fixed experimental conditions, the transformation probability for each organism is proportional to the surrounding DNA concentration [33].

Medium. Divalent cations (Ca^{2+}, Mg^{2+}) interact with DNA [34], and therefore they should generally be avoided. Hyperosmolarity increases the flow from the medium into the organism, and thus generally improves transformation efficiency [35], but to a limited extent, because substantial influx of salts has a detrimental effect on most organisms. Addition of an osmoprotectant into the medium can reduce this effect, thus further improving the transformation efficiency [36]. In yeast, electrotransformation efficiency can be improved considerably by chemical pretreatment, for example, with lithium acetate and dithiothreitol [37], or with thiol compounds [38].

Inactivation of microorganisms

Wastewater treatment

Inactivation of microorganisms by electroporation has already been demonstrated in the 1960s and proved to be efficient for increasing the shelf-life of liquid food [39]; the use of electroporation for microbial inactivation is often termed pulsed electric field (PEF) treatment.

Irreversible electroporation is suited for bacterial decontamination of hospital wastewater, and also eradicates antibiotic-resistant strains, thus limiting the spread of such bacteria into the environment, which is of general concern nowadays [40]. Bacterial inactivation at an energy input of ~150 kJ/l can reduce the bacterial population by four orders of magnitude with wastewater temperature remaining below 70 °C, preserving the activity of nucleases that can thus degrade DNA upon its release from electroporated microorganisms and prevent horizontal gene transfer [41]. Moreover, a combination of mild pre-heating to 60 °C and subsequent electroporation has proved synergistic, leading to the reduction of the required treatment energy for efficient disinfection to ~40 kJ/l [42]. This combination was also found to be effective for the inactivation

of Gram-positive strains that are harder to inactivate by electroporation alone. Unlike disinfection with ultraviolet light, to which bacteria readily develop tolerance, it was shown that disinfection with electroporation does not lead to bacteria developing tolerance or resistance to the treatment for at least 30 generations [43]. Upscaling to pilot-scale flow of 400 l/h demonstrated that electroporation as a disinfection technology is also efficient under high mass-flow conditions.

Nonthermal food pasteurization

Success of electroporation as a mechanism of microbial inactivation in foods strongly depends on several factors: electric field strength and duration, energy delivered, electric properties of the treated food, as well as microbial characteristics, including shape, size, cell wall structure and composition, and growth conditions [44]. While yeast and bacterial cells are susceptible to electroporation treatment, bacterial spores are much more resistant to electrical treatment; therefore, applications of electroporation for microbial inactivation largely aim at food pasteurization rather than sterilization [45].

During the past two decades, electroporation as a method of food preservation has found many applications, and a variety of microorganisms have effectively been inactivated in various liquid foods such as fruit and vegetable juices, cider, beer, milk, and soups, and also in semisolid and solid food products [44–46].

In addition, synergistic effects between electroporation and other treatments, for example, nisin, acid, mild heating, low temperature, or high pressure, have been demonstrated [47]. The approach of combining high pressure, ultraviolet light, and electric pulses also appears to hold promise in the inactivation of bacterial spores for which each of these mechanisms separately often fails to achieve inactivation [48].

Extraction of biomolecules

Unicellular organisms

Microorganisms are being recognized as a potential source of diverse biomolecules for industry, pharmacy or medicine. Established processes to extract these biomolecules include mechanical forces or chemicals, which can be detrimental to the structure and/or integrity of extracted biomolecules [49]. Furthermore, after total microorganism disintegration, purification of targeted biomolecules from cellular debris is needed, which is often costly, requiring additional steps in the process. By contrast, extraction by electroporation (electroextraction) is a fast, chemical-free, energy-saving technique, allows rapid microorganism disintegration resulting in debris to be avoided, and is easily upscalable.

Bacteria. Electroextraction of plasmid DNA (pDNA) was long assumed to be inferior in efficiency to the standard extraction method of alkaline lysis, and was thus studied primarily for applications where a small yield suffices; single-pulse electroporation was thus demonstrated as a feasible but suboptimal method for direct pDNA transfer from donor bacteria, from which DNA was electroextracted, into recipient bacteria which were electrotransformed

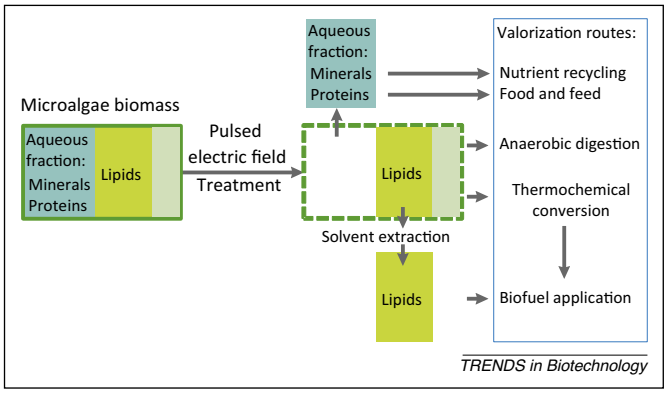

Figure 3. Pulsed electric field treatment of microalgae biomass exhibits fractionating properties. After treatment, the aqueous fraction is released into extracellular medium, whereas lipid droplets cannot pass the cell boundary owing to their size [57]. This allows new processing route combinations for complete microalgae biomass valorization. Proteins and minerals can therefore be recovered before processing of the lipid-rich biomass.

[50,51]. Nevertheless, it was recently shown that, with sufficient optimization, yields with pDNA electroextraction can be comparable or even superior to alkaline lysis [52]. Electroextraction is also applicable for obtaining bacterial proteins [53] and lipids [54]. For each type of molecule, selective size-specific extraction of molecules is generally achievable by adjusting pulse parameters.

Microalgae. Microalgae are currently the most productive biomass feedstock [55], providing ample motivation for developing techniques of extraction of molecules from microalgae, both in batch [56] and flow systems [57,58]. The fractionating characteristics of electroextraction and its high efficiency [59] can overcome current processing hurdles [60,61], in particular for biofuel applications [62,63] (Figure 3), and several companies now utilize electroextraction from microalgae [64]. Electroextraction has been applied to obtain microalgal RNA [65], proteins [66], and pigments [67].

Yeasts. Electroextraction from yeasts was first used for transfer of their DNA into recipient bacteria [68]. Soon afterwards, extraction of proteins was described [69]. A broad range of sizes of functional proteins can be electroextracted, with the yield being dependent on electric field parameters and medium composition [70]. Protein electroextraction was also demonstrated using reversible electroporation, thus preserving yeast viability [71].

Multicellular organisms

Grape. Electroporation of crushed grapes enables fast processing without an adverse influence on taste because the temperature of the mash increases by at most several °C [72]; the crushed grapes are first pumped though the electroporation chamber and are then stored for several hours for extraction to proceed. Combining electroporation with subsequent fermentation on grapeskins gives a more intense color, while combining electroporation with subsequent maceration yields an increased content of polyphenolic compounds in the wine [73]. For white wines, a lighter character of the wine is usually desirable, but for some white grape varieties, electric pulses can be applied

advantageously to achieve a more complete extraction and a more intense taste. Although electroporation also releases more tanning substances, must and wine have less acidity as a result of chemical buffering, resulting in a slightly smoother taste. More nitrogen available to the yeast in the must also helps to prevent the untypical aging note of the wine [74].

Sugar beet. Electroporation of sugar beets enables considerable saving of energy. Conventionally, sugar beet tissue is disintegrated thermally, typically at a temperature of approximately 72 °C, to prepare the cossettes for subsequent countercurrent extraction of the sugar. Treatment by pulsed electric fields (PEF) replaces thermal disintegration by electroporation, with a required energy of ~1–1.5 kWh per ton of sugar beet tissue [74]. Although such treatment can be performed at ambient temperature, in an industrial process the cossettes need to be kept after the exposure at a temperature of at least ~60 °C to prevent mesophilic bacteria from growing, and an inverse temperature profile can be applied advantageously during countercurrent extraction by increasing the temperature from ~60 °C to ~80 °C, the temperature level for the evaporation stages after extraction. Electroporation-assisted extraction (Figure 4) results in a purer juice (because less water is required for extraction) and lower energy consumption during the evaporation stages.

Biomass drying

Efficient drying contributes significantly to energy savings in electroporation-assisted sugar beet processing. After extraction, cossettes are pressed for additional juice removal and dried for use as animal feed. Combining exposure to high-voltage electric pulses with alkaline extraction results in increased dry matter content of the cossettes – from 35% to 40% after pressing [74]. Adding lime milk to the cossettes for alkaline extraction immediately after electroporation strengthens the cell walls and thus fosters extraction of juice during pressing. As a consequence, less evaporation energy is required in subsequent high-temperature drying. In the conventional process of sugar production, lime milk is only used for purging the juice. The alkaline milieu associated with lime milk also reduces corrosion of steel tubes and increases their lifetime.

Dry biomass is also required in fuel production from energy crops. However, energy-efficient drying also allows fresh green biomass to be used. As with sugar beet, drying of green biomass might be carried out by combining electroporation, pressing, and drying in an oven. Electroporation-based treatment of green biomass can be performed in an electrified press; mechanical force is applied before and during pulse application, establishing electric contact to the electrodes through extracted juice without the need to add water [75]. When drying the biomass in an oven, electroporated material dries 2–3-fold faster than non-porated material, not only because of decreased water content after pressing but also because of enhanced diffusion of the vapor as a result of cell disintegration [76].

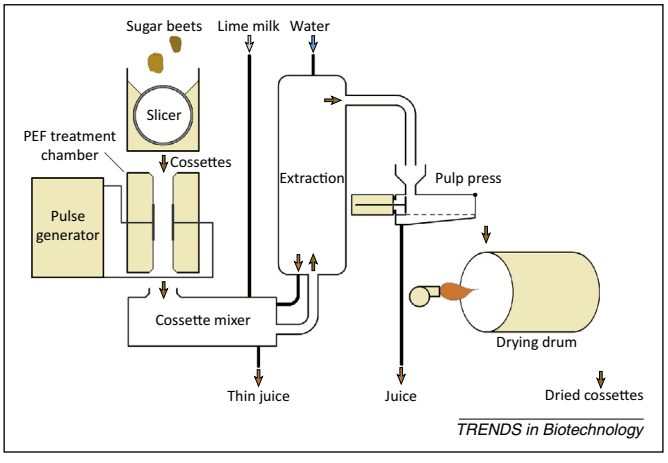

Figure 4. Simplified processing steps for electroporation of sugar beets and alkaline extraction. Washed sugar beets are sliced. The cossettes are immersed in water and treated with a pulsed electric field (PEF) in an electrode system inside the PEF treatment chamber. Liming is carried out inside a cossette mixer. The cossettes are then transported through an extraction tower. Water is added and sugar is extracted from the cossettes using a countercurrent extraction method. Sugar is refined from the thin juice by the conventional processing steps of juice evaporation, crystallization, and centrifugation. The extracted cossettes are pressed to remove additional juice, and fed into a dryer. The dried cossettes are used for the production of animal feed.

Applications of electroporation in microfluidic systems

In the applications described above we often aim to upscale to the industrial and/or clinical level, but there is also motivation for applications of electroporation to sub milliliter samples, with the setups mostly based on microfluidic chambers (lab-on-a-chip devices) in which the electrodes are often designed for multifunctionality, such that electroporation is combined with electrically based analytical processes such as dielectrophoresis, electrophoresis, electro-osmosis, and/or electrochemical analysis [77–79]. Applications can roughly be classified as described below.

Fractionation and/or selective inactivation

If electroporation is preceded by an electrically generated force acting differently on cells of different types and/or sizes, this allows heterogeneous samples of cells to be fractionated and the desired fraction (type) of cells to be selectively electroporated; thus, dielectrophoresis was used to separate leukemic cells from erythrocytes, with the latter being subsequently irreversibly electroporated, leaving only the leukemic cells viable and available for further analysis [80]. If electroporation is followed by a selective electrically generated force, porated cells can be separated from non-porated cells; thus, it was demonstrated that irreversibly electroporated cells can be separated from reversibly electroporated and non-porated cells by dielectrophoresis to more than 90% purity [81].

Extraction for analysis of intracellular contents

While DNA extracted by electroporation can be amplified *in vitro*, other biomolecules cannot, and highly sensitive methods are needed for their detection and particularly quantification. Microfluidic applications of electroporation are performed in suitably small volumes and, combined with on-chip analysis of the extracted molecules, they are able to rapidly and efficiently quantify biomolecules even in very small samples. Thus, a microfluidic combination of electroporation and mass spectrometry

based on electrospray ionization was used to quantify hemoglobin released from single erythrocytes [82].

Selective and/or enhanced transformation

Compared to classical electroporation cuvettes, the optimized channel geometry and microelectrode architecture of microfluidic devices result in less heating and electrolysis, and consequently a higher survival rate of the electroporated cells. Thus, it was recently reported that transformation efficiency in thick-walled microalgae can be improved by two orders of magnitude by electroporating them in an optimized microfluidic chamber rather than in cuvettes [83].

Concluding remarks and future perspectives

Devices for large-scale electroporation comprise one or more pulse generators connected to an electrode system for continuous pulse delivery to a mass flow; both must be carefully designed to achieve desired results [84]. Pulse generators equipped with semiconductor switches in series configuration [85] or in Marx configuration [86], low-scatter spark gap switches in self-breakdown mode [87], and spark gap switches triggered by a semiconductor-based trigger generator [88] are in use and under continuous development. For parallel configuration of Marx generators, over-voltage triggering enables long-term operation of spark gap switches without additional wear [89]. Pulse circuits for rectangular, aperiodically damped, or strongly damped oscillating pulse shapes are applied [90]. The electrode configuration of the treatment chamber is selected with respect to mechanical and electrical properties of the processed mass, and to the pulse circuit grounding scheme [74]. For energy-efficient operation by automatic adjustment of applied energy, measurements of the processed mass impedance can be used to assess the degree of changes caused by electroporation [91–93], but this approach is of limited resolution in materials with high electrical conductivity such as cell suspensions [94]; other monitoring methods allowing real-time adjustments of exposure conditions have therefore been explored.

Integration of electroporation into an existing production line or process must be carefully planned because the cost of required changes may differ considerably for different designs, and may even prove to be unacceptable. When rapid processing is required, introduction of a new step involving electroporation may require adjusting other steps of the process to achieve optimal results [95].

All these considerations point to a need for better knowledge and deeper understanding of electroporation as well as of its effects on the permeability of the cell membrane and cell wall, not only in cellular aggregates but also in intact tissues. Extraction, in particular for large molecules, is inherently limited by the presence of a cell wall, and the same is true for fluid filtration in tissues [96]. Thus, forthcoming research efforts will largely focus on the influence of the cell wall and tissue structure, and on new processing combinations for improving mass transport.

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