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Pulsed electric field: An emerging pretreatment technology in a biogas production

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This review focuses on current status of pulsed electric field (PEF) technology and its implementation in biogas production. First, basic principles of PEF and a schematic overview of typical PEF processing system were provided. Thereafter, lab- and pilot-scale PEF pretreatments of sludge with subsequent anaerobic digestion (AD) were provided. Furthermore, PEF technology, as an emerging technology for the lignocellulose (LC) pretreatment in biogas production which is still predominantly used at lab-scale, was outlined. Eventually, conclusion together with future perspectives and challenges were outlined.

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1. Introduction

The concept of green chemistry has gained increasing interest as a possible approach to the challenge of developing environmentally friendly technologies that use raw materials more efficiently, eliminates wastes and avoids the use of toxic and hazardous materials. Recent studies report that AD is an efficient technology which combines biofuel production and sustainable waste management along with food industry technologies (like microwaves, ultrasound, gamma ray, electron beam, pulsed electric field, high hydrostatic pressure, and high pressure homogenization) to enhance production and quality of biogas as a green chemistry concept (Hassan et al., 2018). As one of such technologies, pulsed electric

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field (PEF) (electroporation or electropermeabilization), has received increasing attention throughout the past several decades for extraction of molecules from cells, insertion of proteins into the cell membrane, induction of cell–cell or cell-vesicle fusion resulting in viable hybrids. It is known to nonselectively increase the uptake of drugs or genetic material into cells, to fuse individual cells with tissue, to initiate targeted necrotic or apoptotic cell death, to induce intracellular effects such as release of intracellular calcium, and modify the texture and viscoelastic properties of plant tissues (Golberg et al., 2016; Rems and Miklavčić, 2016). These tremendous developments in the fundamental understanding of PEF impact on cells suggest that PEF could also become an essential method for energy-efficient technologies.

PEF is non-thermal and less energy-consuming method compared to conventional thermal processes which provides more efficient energetic utilization of plant cell wall compounds and can, therefore, lead to more efficient AD process (Golberg et al., 2016). Although high intensity PEF treatments have minimal thermal effects (temperature usually does not exceed 40 °C (Gabrić et al., 2018)), the temperature of the treated medium increases as the result of Ohmic heating, and is called joule heating. An Ohmic heating process intent to convert electric energy into thermal which results with rapid heating of the treated substrate (Jaeger et al., 2016). However, if needed, cooling system can be attached to the usual equipment (Gabrić et al., 2018).

Effect of PEF on biogas production has not been studied to a large extent (Lindmark, 2014). Mainly, PEF has been so far investigated by using liquid and semi-liquid substrates, such as wastewaters or sludges. The effect of PEF on the degradation of solid, LC-type of materials is relatively unexplored. However, the results of existing studies suggest that PEF improve cells permeability and therefore release soluble components, and disintegrate complex organic molecules into simpler forms making them more available for being the substrates for biological reactions (Safavi and Unnthorsson, 2017a; Liu et al., 2019). Additionally, it is proved that the AD is faster and retention times are shorten when PEF is applied in a pretreatment step (Golberg et al., 2016).

PEF technology is mostly explored for the needs of the food industry. However, during many years technical limitations (lack of reliable and viable industrial equipment) impeded the exploitation of PEF at an industrial level (Sack et al., 2010; Toepfl, 2012). PEF has many advantageous in comparison to thermal treatments, because it inhibits pathogenic and spoilage microorganisms. At the same time, it significantly improves the product quality (preserving color, aroma, flavor and nutrients) without generation of by-products, and resulting with extended shelf life (Haberl et al., 2013; Jin et al., 2015). By permeabilizing cell membranes, PEF enables tissue softening and enhance mass transfer (Rahaman et al., 2019) for biorefineries feedstock development through gene electroporation, dehydration, extraction of high added-value products from waste, LC biomass, and microalgae, extraction of molecules from bacteria and yeast and biogas production (Golberg et al., 2016). Furthermore, PEF can be used for pasteurization of liquids (e.g. juices, dairy products and soups) (Kurcevskis et al., 2019). PEF is rather applied for the production of liquid and semi-liquid food products like fruit juices and smoothies, for pretreatment of vegetables and tubers before cutting, blanching, or extraction of valuable or desired compound, in dairy industry for preservation of cheese milk or whey protein (Buckow et al., 2013; Moller and Dőnitz, 2018).

Beside all the advantages, PEF technology has a number of potential limitations. The most important limitation lies in the high costs which are mostly related to the initial capital investments. A pilot plant-size unit may cost around $250,000, while units that are manufactured for industrial scale are available at prices that range from $45,000 to $2,000,000 (Vega-Mercado et al., 2007; Alirezalu et al., 2020).

PEF is ineffective at deactivating spores and viruses (Kempkes, 2010) and is generally not suitable for solid foods. Moreover, treated products must have lower electrical conductivity whereas the presence of bubbles in the treatment medium leads to non-uniform treatment as well as operational and safety problems (Yogesh, 2016). Additionally, particle size should be less than the gap of the treatment region in the chamber in order to maintain an appropriate treatment (Syed et al., 2017).

A number of pretreatment methods (such as different mechanical, physical and biological methods, and their various combinations) have been investigated aiming to enhance biogas production with minimal economic costs, minimal energy consumption and without negative environmental impact (Amin et al., 2017; Hernández-Beltrán et al., 2019; Abraham et al., 2020). All of them have their own advantages and disadvantages/limitations. In comparison to other pretreatment methods, PEF offers the advantages of low energy input since it does not require heating (Siemer and Toepfl, 2020); it does not require chemicals to be added for pretreatment which makes it environmentally friendly and safe for the operation (Rewagen, 2016); and it is short time process (treatment time from microsecond to second) (Joannes et al., 2015).

The purpose of this review was to summarize the state of the art and to present future perspectives related to the application of PEF technology in biogas industry.

2. Basic aspects of electroporation induced by pulsed electric field

Even though PEF technology has long been used in molecular biology (Weaver and Chizmadzhev, 1996) and, in recent times, is successfully used in medicine, biotechnology and mainly food processing (Donsì et al., 2010; Golberg et al., 2016) there are still gaps in understanding the phenomenon of electroporation and its basic molecular and cellular mechanism. It is believed that it is based on the so called dielectric breakdown theory which is based on the selective damage of the biological membrane which is almost entirely consisted of cholesterol and phospholipids (De Vito, 2006). Each phospholipid molecule, which is described as amphiphilic, is consisted of a glycerol hydrophilic (polar) part and two hydrophobic hydrocarbon (nonpolar) parts (Chen et al., 2006; Wayne, 2010). Hydrophilic part, known as "head", is rather compact. Two hydrophobic parts are more elongated and are called "tails". In that stable bilayer, nonpolar tails are oriented inward, and the polar heads outward. Because of nonpolar interior, this layer is almost impenetrable barrier for polar molecules (Chen et al., 2006; Kotnik et al., 2012). However, under certain conditions (sufficiently high temperature or surface tension, or both) water and some monoatomic ions can permeate through it. This permeation can be induced by thermal and mechanical fluctuations and can be attributed to the formation and rapid resealing of very small aqueous pores in the lipid bilayer. The pores can be formed without external electric field acting on the membrane, but they are inherently unstable (Kotnik et al., 2012).

Biological membranes are considered as capacitors filled with a dielectric material of low permittivity, and they maintain an electrochemical gradient at both membrane sides. This gradient occurs because of the excess of negative ions, which are accumulated at the inner surface of the membrane and, to an equal number of positive ions accumulated outside the cell. Across the cell membrane, a transmembrane potential called the resting potential, is formed. After being exposed to a sufficiently high external electric field,

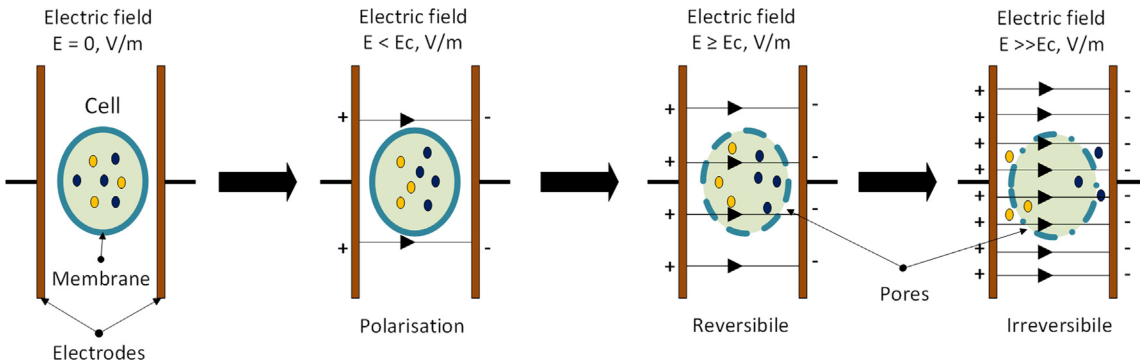

Fig. 1. Accumulation of opposite charges on both sides of cell membrane due to exposure to electric field (polarization) which creates reversible (pore resealing) or irreversible (cell death) effects – electroporation. *Ec* – critical electric field strength. (adapted from: Korma et al., 2016).

membrane ions migrate toward the membrane walls what results with the accumulation of the free charges at the both sides of the membrane. The accumulation of membrane charges results with an increase of the potential difference across the membrane. The electric field induces an additional transmembrane potential, larger than the membrane's natural potential, which is unevenly distributed over the surface of membrane. Breakdown of the cell membrane (also known as electroporation) (Fig. 1) occurs when the overall transmembrane potential (sum of the induced and the resting potential) reaches a threshold value, the critical transmembrane potential upon which micropores are formed on the membrane increasing permeability that can lead to membrane rupture (De Vito, 2006; Ricci et al., 2018). Depending on electric field strength (*E*) and treatment intensity (*TI*), rupture of membrane may be reversible or irreversible (Bot et al., 2017). In the case, when increase in membrane permeability is only temporary and the membrane regains its initial selective permeability when electric field is terminated, electroporation is said to be reversible. Reversible electroporation occurs in three stages - pore formation, pore expansion and pore reasiling. It is mostly studied and used in biotechnology for the transfer of genetic materials (DNA) inside bacterial cells as well for improvement of fusion of cells (De Vito, 2006; Golberg et al., 2016). Otherwise, if the cell dies, the breakdown of membrane is said to be irreversible.

Parameters that have influence on the *E* which is necessary to achieve electroporation can be divided into: (a) process parameters (pulse shape, duration, frequency etc.), (b) physico-chemical parameters of the cell (conductivity and ionic composition of the extracellular medium, cell density, osmotic pressure etc.) and (c) cell parameters (type, size, shape and orientation) (Saulis, 2010; Bhat et al., 2018).

There are different tools available for analyzing the impact of electroporation on cellular and tissue levels. Numerous substances serve as electroporation indicators such as fluorescent dyes (the most frequently used), color stains, magnetic nanoparticles, functional molecules such as cytotoxic bleomycin, and nucleic acids which are mostly used in electrochemotherapy. Detection of fluoroscent dyes uptake can be determined by fluorescence microscope, spectrofluorometry or flow cytometry, while color stains uptake can be determined by simple light microscope or spectrophotometrically. Magnetic nanoparticles can be detected by magnetic resonance imaging (MRI), various staining protocols or magnetic force microscopy (MFM), or can be separated from nonlabelled ones in a magnetic field. Beside exogenous molecules and cell leakage, physical and chemical methods, such as conductivity impedance measurements, voltage clamp methods or cell swelling, can be also used for detection of electroporation (Napotnik and Miklavčić, 2018).

Moreover, other techniques can be used to understand the mechanism of electroporation such as electron and atomic force microscopy for visualization of damages in cell membranes, selective medium plating technique for membrane damages evaluation,

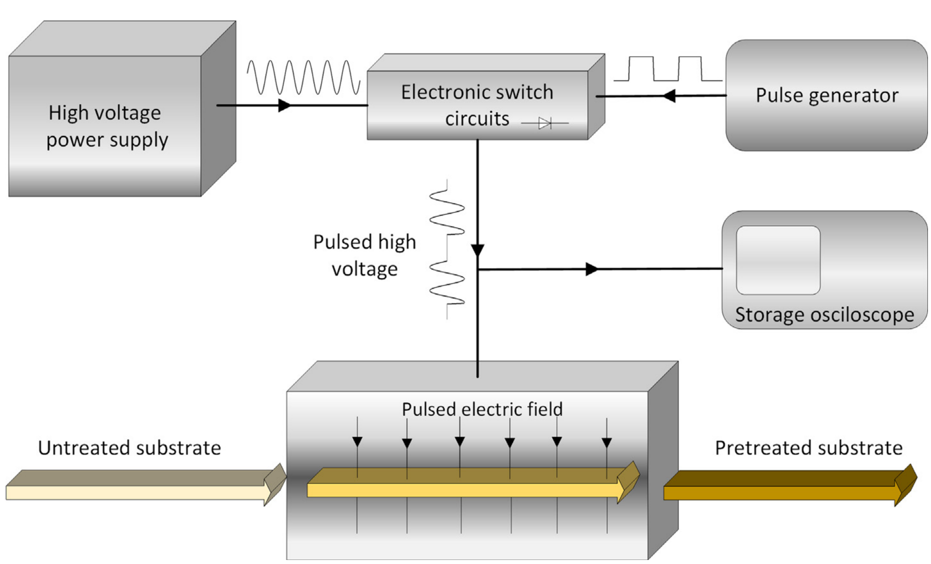

Fig. 2. Typical PEF processing system (guidance, control and pretreatment).

osmotic response measurement, and Fourier transform infrared spectroscopy (FTIR) (García-Gonzalo and Pagán, 2016).

3. Pulsed electric field (PEF) system design

In this chapter, typical PEF system designs and studies performed to enhance efficiency and increase cost-effectiveness of PEF treatment are elaborated. Since PEF treatments have been most widely studied for applications in food industry, the major number of listed studies is addressed to food substrates.

A typical PEF processing system (Fig. 2) is consisted of a high-voltage power source, a function generator, a switching circuit, an oscilloscope and a treatment chamber (Kumar et al., 2009; Mohamed and Eissa, 2012).

During pretreatment, the substrate is placed between two electrodes in a treatment chamber. The treatment area and gap between the electrodes is preferred to be small in order to have an uniform and strong electric field (Kumar et al., 2009). The substrate is separated by an insulating material which protects it from undesired effects of electrodes and minimizes Ohmic losses. Most frequently used insulating materials are polythene, polypropylene, polysulfone, plexiglas and PVC (De Vito, 2006; Ricci et al., 2018). The proper design of geometry and size, as well as construction material of treatment chamber, are very important because, apart from assuring a uniform distribution of electric field and prevention or elimination of air bubble formation (when treating pumpable substrates), it also affects the specific energy input ($W_{spec.}$) during processing and, thus, the temperature increase (Buckow et al., 2011; Sahu, 2014). Treatment chambers can be used for batch or continuous processes. Classification of batch and continuous PEF processes are presented in Table 1 together with their specifications.

The ongoing issue in continuous PEF pretreatment processes is uniformity of electric field distribution and uniform electroporation of entire substrate, stable flow characteristics, the extent of the temperature increase, as well as uniform velocity distribution. Because in practice, this is difficult to achieve simultaneously, some optimization of process parameters must be made (Meneses et al., 2011; Kandušer et al., 2017). Unlike continuous processes, in batch treatment chambers uniform treatment conditions can be assured and delivered equally to the entire substrate (Kandušer et al., 2017).

Schroeder et al. (2009) developed and validated a specific computational fluid dynamics model describing the stationary flow, the electric field and the temperature distributions inside a pilot-scale PEF treatment chamber with co-linear electrodes arrangement for pasteurization of liquid foods. The standard chamber geometry showed a very inhomogeneous electric field and poor mixing pattern of the fluid leading to local hot spots and a strong temperature increase. A reduction of the insulator's inner diameter combined with rounded edges or an elliptical shape significantly improved the uniformity of the electric field and the turbulence pattern of the flow. Numerical simulations showed that small modifications of the insulator geometry can reduce the temperature increase in the treated media by up to 70% which can result in more efficient process. The model can be used for estimation of impact of various designs of treatment chambers on the process safety and treatment

Table 1
Clasification and specifications of batch and continuous PEF pretreatment processes.

Batch processes	References	Continuous processes	References
• Mostly used in laboratory researches. Investigation of the basic PEF mechanisms.	Kandušer et al., 2017	• Preferred for pilot plants and industrial scale operations.	De Vito, 2006; Huang and Wang, 2009
• Developed and used by various research groups. Their researches gained to the modifications that made PEF processing easier, more convenient, more reliable and more cost-effective.	Sahu, 2014	• Problems related to non-uniform distribution of the electric field in the treatment zone.	Kandušer et al., 2017
• Although mainly used for the treatment of liquids, suitable also for treatment of solid and semi-solid substrates.	Mohamed and Eissa, 2012	• Treatment efficiency and homogeneity can be predicted by applying numerical simulation techniques that are becoming more and more popular with the progress of computational capabilities.	Wölken et al., 2017
• The most common types of the batch treatment chambers available in the market: U-shape, parallel plate, disk-shape, wire cylinder, rod-shape and sealed.	Huang and Wang, 2009; Sahu, 2014	• The most common types of treatment chambers: concentric cylinder, concentric cone and co-field (co-axial or co-linear).	
• Parallel plate configurations have the simplest design, provide the most uniform distribution of the electric field and assure homogeneous treatment.	Meneses et al., 2011; Puértolas et al., 2012; Toepfl et al., 2014; Fredriksson, 2018; Shorstkii et al., 2020	• Co-field treatment chamber designs provide a near-optimal balance between the liquid substrate flow and field requirements, with a simple and easy to manufacture design and are favored over most designs.	Barbosa-Canovás and Altunakar, 2006; Kempkes, 2017
• Parallel plates design best suits for treatment of bulk substrates (e.g. apples and potatoes).	Kempkes, 2017	• In a co-axial treatment chamber (application restricted to liquid substrates) the substrate is placed between two cylindrical electrodes (high-voltage internal and ground external). The electric field is distributed in descendant order from the internal toward external cylinder which results in non-uniform distribution of electric field. High current flow and a low resistance of the treatment chamber is caused by large effective area of the electrodes.	Meneses et al., 2011
		• Co-linear treatment chamber is most commonly used. An insulating spacer keeps a hollow high voltage and grounded electrode with a circular inner hole on a defined distance. The E, which is not homogenous, depends strongly on the insulator geometry. It is possible to obtain different E distributions by modification of the insulator geometry which can be simulated with numerical software.	

uniformity, as well as for better understanding of the critical process points. Similar research was conducted by Sarathy et al. (2016) who developed a model to enable simulation of the interdependent flow, electric field and temperature distributions throughout the fluid flowing through the wastewater sludge treatment chamber. The model vas validated by comparison of parameters predicted by model and measured parameters.

The E is not homogeneous and strongly depends on the insulator geometry. By modifying insulators geometry, it is possible to obtain different E distributions that can be simulated with numerical software (Gerlach et al., 2008) which was confirmed by the study of Buckow et al. (2011) who performed a systematic study of pilot-scale PEF treatment chambers (more than 150 dimensions and different insulator geometries) with co-linear electrode configuration. A high voltage pulse generator, which can either be an ordinary source of direct current (DC) or a capacitor charging power supply with high frequency alternating current (AC) inputs, feeds high voltage repetitive pulses (usually 1–20 μs) of desired voltage, shape and duration to the switching circuit. The switching circuit is turned on when the pulse is applied to it and transfers the high voltage (15–80 kV cm^{-1}) supplied by the power supply across the sample holder (Kumar et al., 2009; Mohamed and Eissa, 2012; Picart-Palmade et al., 2019).

It is of great importance to include a control system in the design of pulse generators to protect the device from unexpected faults (e.g. overheating of the switch) because electrical components can be seriously and irreversibly damaged when samples with too high or too low resistivity are processed. Control system is consisted of two major devices - a temperature probe and an oscilloscope which measures the voltage across the treatment chamber and shows the output voltage (De Vito, 2006).

3.1. PEF processing parameters

The most typical process parameters (Table 2) that characterize PEF technology are: electric field strength (E), treatment time (t), pulse shape, pulse width (τ), number of pulses (n), pulse specific energy input ($W_{spec.}$), and pulse repetition frequency (f) (Raso et al., 2016). The E, pulse width and number of pulses (or treatment time $t = \tau \cdot n$) are the main process parameters that define the PEF TI (Donsì et al., 2010).

A various studies of PEF process parameters can be found in literature. Few researches have addressed the problem and emphasized that with adjusting process parameters it is possible to achieve the uniformity of electric field distribution. Flisar et al. (2014) pointed out that with increasing the number and frequency of pulses delivered to substrate the uniformity of electric field distribution can be assured. Additionally, to achieve that it is important to assure the stability of the circuit for generating pulses and an adequate supply of energy for pulse generator. The effect of monopolar and bipolar shaped pulses was studied on the yield of apple juice extraction by Brito et al. (2012). Energetic consumption had a threshold where higher energy inputs did not result in higher extraction of juice, while on the contrary bipolar pulses demonstrated higher efficiency. There were no significant differences in total soluble dry matter and absorbance results between monopolar and bipolar pulses application, but all values were within the limits which are proposed for apple juice intended for human consumption.

Likewise, Evrendilek and Zhang (2005) reported that there was no significant difference between mono and bipolar pulses on the inactivation of *E. coli* inoculated into apple juice. However, there was a significant difference between mono and bipolar pulses in the yields of skim milk, whereas bipolar pulses were significantly more efficient. Lebovka et al. (2001) showed that apple tissue which was treated with pulse frequencies between 0.02 and 100 Hz resulted in more tissue damage in comparison to pulse frequencies beyond 100 Hz which did not lead to higher tissue damage. Ersus et al. (2010) conducted a research in which they studied the influence of electrical pulse protocol parameters on onion tissues cell rupture in order to improve fundamental understanding of the process and to enhance the electroporation of plant tissues. E, total pulse duration, pulse width, and frequency effects were determined as functions of pulse protocol regarding their cell damage effects. E up to 0.5 kV cm^{-1} increased the damage efficiency but no significant difference in efficiency beyond this field strength was determined. Larger pulse widths increased tissue disintegration degree of at a constant pulse number. Higher PEF efficiency was achieved with larger number of pulses but shorter pulse widths at a constant total treatment time. Lower frequencies resulted with greater degree of disintegration at constant number of pulses. A similar study was conducted by Aguiló-Aguayo et al. (2015). They studied the effects of E, number of pulses, pulse frequency and pulse width on the extraction of polyacetylenes from carrot slices. Maximal extraction yields of polyacetylenes were achieved after applying PEF at 4 kV cm^{-1} with 100 number of pulses of 10 μs at 10 Hz. Likewise, Elez-Martínez and Martín-Belloso (2007) performed a study in which orange juice and cold vegetable soup (gazpacho) were submitted to high intensity PEF while process parameters (E, treatment time, pulse frequency, width and polarity) were alternated in order to evaluate the effect of PEF on vitamin C retention and antioxidant capacity of the both products. They compared the results with those obtained after standard pasteurization process. They came to the conclusion that pulses applied in bipolar mode, as well as a lower E, treatment time, pulse frequency and width, led to higher levels of vitamin C retention. If compared to pasteurized products, PEF-treated orange juice and gazpacho always resulted with higher vitamin C retention, while no significant difference in antioxidant capacity between PEF-treated and untreated products was no detected.

Pulse specific energy was studied by Buchmann et al. (2018) who compared the flow field in continuous PEF processing and energy input distributions in PEF co-linear and parallel plate treatment chambers for the microalgae processing. To make the results as comparable as possible, a co-linear treatment chamber with a 4 mm gap between the insulators was compared to a parallel plate treatment chamber with a 4 mm electrode gap. All other parameters like inlet velocity, electric potential, viscosity, pulse width, pulse repetition rate, and conductivity of the medium were kept equal for both treatment chambers. $W_{spec.}$ calculations were examined to define and understand what made up the in homogeneities in both treatment chamber geometries and what was different between the two geometries.

Depending on process parameters (E and $W_{Tspec.}$), PEF applications can be divided into different types: microbial inactivation (15–40 kV cm^{-1} and 40–1000 kJ kg^{-1}); improvement of mass transfer in plant or animal cells (0.7–3.0 kV cm^{-1} and 1–20 kJ kg^{-1}); sludge disintegration (10–20 kV cm^{-1} and 50–200 kJ kg^{-1}); reversible electro-permeabilization of biological cells for DNA transfer (0.7 kV cm^{-1} and 1–10 kJ kg^{-1}); induction of stress response (0. 5–1.5 kV cm^{-1} and 0.5–5 kJ kg^{-1}) (Oey et al., 2016; Picart-Palmade et al., 2019).

In literature, process parameters and electric field pretreatment protocols are often poorly and unevenly described which can be a result of differences in PEF equipment and treatment conditions. The lack of a standardized way of reporting treatment conditions and protocols prevents the conducted research to be reproduced in other laboratories. Therefore, Raso et al. (2016) published a paper in which they provided recommended guidelines on the key information that should be reported in all the researches dealing with the investigation of PEF in the field of food technology and biotechnology. The guidelines are proposed to simplify the com-

Table 2
Typical PEF process parameters.

Parameter	Symbol	Unit	Characteristics	References
Electric field strength	E	kV cm^{-1}	• the most critical process parameter • has influence on the degree of cell membrane electroporation • the intensity of E is determined by the amount of voltage discharged (in kV) into the chamber and the distance (in cm) between the two electrodes, where the substrate is placed • with increasing the gap between electrodes a higher voltage is required to obtain the desired critical electric field corresponding to the membrane breakdown • the higher the field strength, the larger the electroporation phenomenon	De Vito, 2006; Kumar et al., 2009; Álvarez and Raso, 2017; Leong and Oey, 2019
Treatment time	t	s	• function of the pulse width and the number of pulses applied • a longer effective treatment time can be achieved by increasing the pulse width • the number of pulses causes an increase of treatment time	Toepfl et al., 2005; Puértolas et al., 2012; Ricci et al., 2018
Pulse width (pulse duration)	τ	µs	• defined as the amount of time by which a pulse is held at an effective voltage • pulse duration is determined upon pulse shape applied	Ricci et al., 2018
Pulse shape	–	–	• exponential decay and square waveform are the commonly used pulse shapes • square waveform geometry has been determined to be the most suitable to maximize the effect of PEF treatment • for square waveform, effective pulse width is measured at 50% of peak voltage since an ideal square pulse shape can be hardly achieved • for exponential decay pulses, the time before the electric field decreases to 37% of peak voltage defines the effective pulse width	Reberšek, 2017; Ricci et al., 2018
Number of pulses	n	–	• pulses can be of constant polarity (monopolar or unipolar pulses) or alternate polarity (bipolar pulses) • the most commonly applied are monopolar exponential decaying pulses, and monopolar and bipolar square wave pulses • monopolar pulses retain positive charge and involve no reversal of polarity in the electric field • bipolar pulses are characterized with instant reversal of charges which rapidly reverse the electric field orientation	De Vito, 2006; Leong and Oey, 2019
Pulse frequency	f	Hz	• the number of pulses applied by unit of time • highly influence the effectiveness of PEF treatment on cell tissue	Puértolas et al., 2012; Leong and Oey, 2019
Pulse specific energy input	$W_{spec.}$	kJ kg^{-1}	• depends on the voltage applied, treatment time, and resistance of the treatment chamber • calculation of $W_{spec.}$ is important because it allows a comparison of PEF treatment efficiency with other technologies and provides estimation on the energy consumption and processing cost	Heinz et al., 2001; Leong and Oey, 2019
Treatment intensity	TI	kWh m^{-3}	• utilized to combine the effects of E, pulse width, frequency, and hydraulic residence time, as well as to compare the results obtained using different electric pulse protocols and PEF devices • It is calculated using the following equation: $TI = (K \bullet (V^2 \bullet \tau \bullet f \bullet \sigma \bullet \mathrm{HRT}))/L^2$, where K is the constant for unit conversion, V is the voltage applied, τ is the pulse duration, f is the pulse frequency, σ is the sample conductivity, HRT is the residence time in the treatment chamber, and L is the length of the treatment chamber	Salerno et al. (2009)

parison of data, to create a reliable basis for a better understanding on the influence of different factors on the PEF efficiency, as well as on the involved mechanisms. The guidelines might also help to new researchers to obtain repeatable, reproducible and free from methodological errors data.

Authors recommended the most relevant information that should be provided in manuscripts: an appropriate description of the PEF generator, treatment chamber and auxiliary devices (e.g., voltage, current and temperature probes, pump, and heat exchangers). In order to provide a proper comparison between data of different authors, E and total specific energy input $W_{Tspec.}$ should be chosen as process parameters that describe the TI. Moreover, initial voltage applied, pulse shape, pulse width, number of pulses or treatment time, frequency, pulse protocol, initial and final temperature for batch processes, and mass flow, residence time, inlet and outlet temperature for continuous flow chamber, are process parameters that should be also specified.

4. PEF in biogas production

Biogas production via AD process is one of the most efficient technologies for providing clean and renewable energy from organic wastes (González et al., 2020). All kinds of solid or liquid organic biomass wastes such as wastewaters from different origin, organic fraction of municipal solid waste, sewage sludge, animal manures, agricultural crops and residues, microalgae and food waste can serve as AD substrates (Rasapoor et al., 2020). AD (Fig. 3) depends on bioavailability of molecules bound within cells, clumps, and other conglomerates. The slowest and the rate limiting step is the hydrolysis where main high molecular weight constituents of organic substrate (proteins, carbohydrates and triglycerides) are degraded into simpler dimers and monomers (amino acids, monosaccharide and disaccharide sugars, and fatty acids) which are available for digestion (Pliquett, 2015). Therefore, extensive research has been conducted on pretreatment methods to accelerate hydrolysis step of complex organic substrates to simpler forms (Lee et al., 2019). Many of these technologies come from the wastewater treatment or bioethanol production and they can be divided in four main groups: mechanical, physical, chemical, and biological pretreatment methods (Kovačić et al., 2017). It is often difficult to assess which ones are worthwhile since their disadvantages are often neglected and costs are rarely considered in research papers. Besides, many methods that look promising at small scale and in batch conditions may not be effective at large

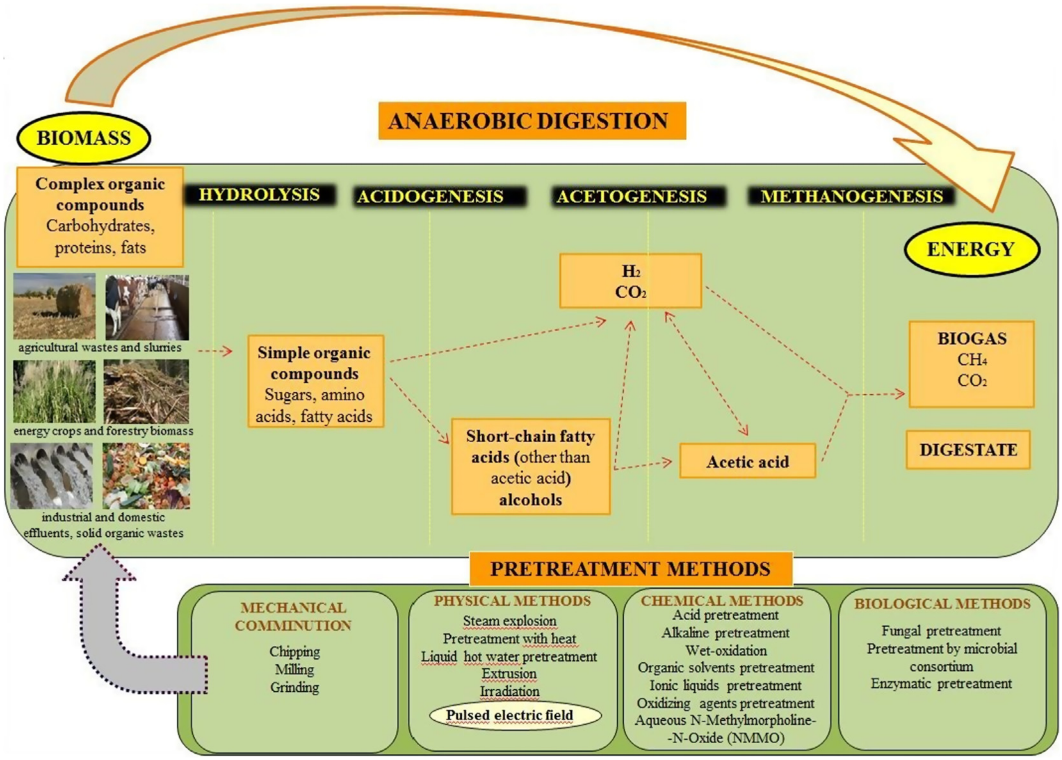

Fig. 3. AD process flow chart and most frequently used methods for pretreatment of biomass.

scale and in continuous or semi-continuous processes (Bochmann and Montgomery, 2013).

In biogas production, PEF has been suggested as an effective method for accelerating the hydrolysis step. The most pronounced effect of the electric field on the raw substrate is the increase of bioavailability of compounds by cracking of cell structures. This yields to the faster AD and a shorter retention time. The optimization of PEF protocols focuses on economic measures like the additional biogas yield with respect to the consumption of energy, the reduction in hydraulic retention time, and the final content of biosolids after digestion. However, process optimization is challenging because of the many variables (e.g. treatment time, *E*, pulse shape and width, pulse duration, number of pulses, pulse frequency, distance between electrodes etc.) that can yield to the highly variable outcome (very low to high biogas/methane (CH_4) yields). Pretreatment parameters should be chosen with respect to the each single bioreactor and substrate (Golberg et al., 2016).

PEF technology has been widely used in wastewater treatment for the effective elimination of organic compounds and nutrients, and reduction of bacteria in contaminated fluids like domestic, hospital and industrial wastewaters before discharge in receiving water bodies. However, PEF technology is fairly new in the sludge treatment (Gusbeth et al., 2009; Kuşçu and Çömlekçi, 2015) while it was only five years ago that it emerged in LC treatments with the aim of biogas production (first available published research dates back from 2014). Therefore, most studies that were conducted assessed the impact of PEF pretreatment on the biogas yield on a laboratory scale with only few determining the net energy gain obtained after anaerobic digestion of pretreated substrate.

4.1. PEF pretreatment of sludge for biogas production

The primary by-product of wastewater treatment plants, waste activated sludge (WAS), is generated during the wastewaters pretreatment process in the primary and secondary clarifiers and must be treated and disposed safely. WAS is hazardous since it contains pathogens and heavy metals as well as a variety of organic compounds that make it renewable energy source (Kuşçu and Çömlekçi, 2015; Demirbas et al., 2017; Zhao et al., 2017; Skórkowski et al., 2018). Although the sludge represents only 1 to 2% of the treated wastewater volume, its management is highly complex (thickening, dewatering, final disposal) and has a cost which usually exceeds 50% of the operating costs of wastewater treatment plants. A number of different physical, chemical and biological methods have been developed for sludge treatment, minimization and stabilization whereas biological process of AD is mainly used worldwide (Luduvice, 2007; Skórkowski et al., 2018).

Three types of sludge should be considered for process of AD: primary sludge (PS), secondary sludge (WAS) and a mixture of both. PS is generated during physical treatment in primary settlers and is easily biodegradable because it contains easily biodegradable polysaccharides and fats. WAS is generated after biological treatment and is less biodegradable and less hydrolysable in comparison to PS, because it contains a diverse and flocculated mixture of active and inactive microorganisms, extracellular polymeric substances (proteins and polysaccharides) and nondegradable organic and mineral matter substances (Lee and Rittmann, 2011; Pinto et al., 2016; Elalami et al., 2019; Lee et al., 2019). The efficiency of AD is often highly limited due to number of factors, such as complex structural, recalcitrant components in sludge, rate-limiting cell lysis, the presence of extracellular polymeric substances and various inhibitory compounds. These limitations often impair digester performance resulting in long retention time, large digester capacity, and consequently high operational costs (Hosseini Koupaie et al., 2017). In order to overcome these issues, different pretreatment methods aim to improve process performance at the bench-scale (greater reduction in VS, release of soluble chemical oxygen demand (COD) and generation of biogas).

The main purpose of pretreatment is to enhance the hydrolysis which is the rate-limiting step in AD of sludge, and consequently enhance biogas production (Skórkowski et al., 2018).

PEF is an innovative technology in the field of biogas production which was adapted to improve performance of the AD process. The earliest available study related to application of PEF pretreatment method in biogas production dates from the beginning of this century. PEF technology acts in the way that it disrupts the structure of sludge aggregates and reduces complex organic molecules to simpler forms making them more bioavailable to anaerobic microorganisms in digester. Subsequently, anaerobic microorganisms utilize this dissolved organic material as substrate for CH_4 generation increasing the digester performance (Lai et al., 2014; Kuşçu and Çömlekçi, 2015). However, little is known about how PEF pretreatment affects microbial communities in anaerobic reactors (Zhang et al., 2009). So far, only one group of authors has studied the effect of PEF pretreatment of sludge on anaerobic microbial community's composition. After their first attempt to study the effect of PEF on microbial population in AD, they concluded that PEF pretreatment has a profound impact on the structure and function of the anaerobic microbial community which correspond to increase in CH_4 generation rate. Their results showed that phylotypes related to *Deltaproteobacteria* and *Spirochaetes* increased in the digester after PEF pretreatment while within the archaeal community *Methanoculleus* shifted from 66% to 32%, and *Methanosaeta* from 22% to 55%. The *Methanoculleus*-related phylotypes shared a sequence similarity of 97% to the cultured species *Methanoculleus thermophilus* and *Methanoculleus marisnigri*, which are known to use H_2, but not acetate for CH_4 utilization. On the contrary, *Methanosaeta* species are acetate-cleaving methanogens, which are in most cases the predominant methanogens in anaerobic digesters (Rittmann et al., 2008). Their subsequent research significantly extend previous findings and revealed that sludge pretreated with PEF consists of more diverse bacterial community and is populated more by phylotypes associated with cellulose fermentation (*Ruminococcus*), scavenging of biomass-derived organic carbon (*Chloroflexi*), and homo-acetogenesis (*Treponema*). The overall activity of the bacterial community was stimulated by addition of more bioavailable organic matter. Therefore, bacterial community became more phylogenetically diverse so that it can take advantage of the added input of bioavailable organic matter and in response to the more efficient utilization of acetate by *Methanosaeta* (Zhang et al., 2009).

The effect of PEF pretreatment on sludge can be analyzed by different techniques. The most common tests to determine the disintegrative effect of PEF is the release of dissolved organic carbon (DOC) or the uptake of COD (Koners et al., 2006a). Salerno et al. (2009) evaluated the impact of PEF *TI* applied to the WAS and pig manure on the conversion of organic solids material to soluble and colloidal forms which are more bioavailable for CH_4 production during subsequent AD. They found that conversion increased steadily for *TI* up to about 40 kWh m^{-3}. The biochemical methane potential (BMP) of treated samples increased significantly: by 80% for pig manure and 100% for WAS after 25 to 30 days in comparison to untreated sample. Authors concluded that PEF treatment has more impact on bioavailability by producing small colloids than producing truly soluble organic material. Lee and Rittmann (2011) performed similar research in which they carried out PEF treatment at average *TI* of 34 kWh m^{-3} which resulted in increase of CH_4 production rate by 33% and COD removal efficiency by up to 18%. Furthermore, Safavi and Unnthorsson (2017b) applied three *TI*s of 15, 30, and 50 kWh m^{-3} to fruit/vegetable slurry and landfill leachate to investigate the influence of pretreatment on CH_4 production. CH_4 production of the landfill leachate and fruit/vegetable slurry increased by up to 44 and 8% with the *TI* of 50 and 15 kWh m^{-3}, respectively. COD removals were increased by up to 100% for landfill leachate and 17% for fruit/vegetable slurry, compared to the untreated slurries. Kuşçu and Çömlekçi (2015) pretreated WAS with PEF to enhance its AD and evaluated it in terms of COD conversion to CH_4. They performed simulations of electric field distributions, conductivities and two different electrode gaps. AD of pretreated WAS resulted in increased CH_4 content by 1.36 fold at optimum pretreatment conditions (σ = 4 mS cm^{-1}, 5 ml min^{-1} of flow rate and V = 54 kV).

Several authors reported microscopic pictures that show the effect of the disintegration on the sludge particles composition very clearly. For instance, Kopplow et al. (2004) and Koners et al. (2006b) stained PEF treated sludge particles with Crystal Violet Sheath Stain which afterwards revealed disturbed edges but untouched inner flocs size structure under microscope. Choi et al. (2006) observed effect of PEF treatment under scanning electron microscope (SEM) and images clearly showed destructed sludge cells which resulted in 2.5 times higher biogas production in batch AD if compared to untreated sludge. On the other hand, Gao et al. (2015) evaluated the effect of PEF on microorganisms found in sludge under optical microscope. They assumed that microorganisms in sludge also undergo a change in membrane structure after PEF treatment. Therefore, they stained microorganisms isolated from treated sludge with trypan blue color and observed that the area inside the cell tends to be blue which indicated that small molecule of trypan has passed through the membrane into cell which indicated that the electroporation phenomena has occurred.

Mostly, PEF has been applied for pretreatment of WAS. However, Ki et al. (2015) pretreated PS with PEF and afterwards evaluated the effect of PEF pretreatment through BMP assay. Moderate improvements in the bioavailability of organic solids in PS were achieved after PEF treatment of PS. COD conversion to CH_4 increased by 8% which is quite low if compared to above mentioned studies with WAS pretreatment. Authors also concluded, after comparison of obtained results, that impact of PEF on PS is weaker than on WAS.

All above-referred studies are lab- and/or pilot-scale studies. They all outlined the potential of PEF for pretreatment of sludges and manures to make them more bioavailable in AD process. The working range of E in those studies was from 5.8 to 30 kV cm^{-1}. All PEF operational settings and parameters of elaborated studies are presented in Table 3 in detail.

Based on these lab- and pilot-scale studies, full-scale studies were conducted and implemented extensively in sludge processing worldwide (Zhen et al., 2017). The world's first known commercial deployment of full-scale PEF technology arose in Arizona, in 2006. It was specially adapted from food industry to overcome the economic, operating and scale up issues in sludge treatment. It is installed at the wastewater treatment plant between the centrifuges and the two mesophilic anaerobic digesters for treatment (V = 20–30 kV) of mixture of thickened PS and WAS. Group of authors (Rittmann et al., 2008) performed a 6-month study which resulted in increase of SCOD by 160% and DOC by 120% in comparison to untreated sludge mixture. The average biogas production increased steadily by more than 40% in comparison to baseline average biogas production throughout 6 measured months, while CH_4 concentration in biogas didn't change (62%). In addition, monthly biosolids content discharged from the digesters decreased by 25–30%. Furthermore, authors provided an example of economic calculation for a specific PEF pretreatment plant based on referred research. They take an average of 380 m^3 day^{-1} of WAS and PS with a VS/TS ratio of 80% generating an average of 6100 m^3 day^{-1} of biogas with an economic value of \$0.28 m^{-3} and disposing of 12,300 dry kg day^{-1} of dry biosolids at a cost of \$0.28 per kg. Without considering any other benefit (like heat recovery or reduced polymer), PEF pretreatment generates an economic benefit of approximately \$540 000 annually net of electric-

Table 3
Operational settings and parameters of lab-scale and pilot-scale studies of PEF pretreatment of different sludges.

Feedstock	PEF pretreatment parameters	Effects of the pretreatment	Reference
WAS from wastewater reclamation plant and pig manure	Cylindrical treatment chamber. Sludge: V = ~10 kV; τ = ~10 µs; f = ~1000 Hz; σ = ~0.1 S m^{-1}; L = ~0.01 m; HRT = 0.01 s; TI = 4.0–19.8 kWh m^{-3}; E = 24.5 kV cm^{-1}. Manure: V = ~ 10 kV; τ = ~10 µs; f = ~ 1000 Hz; σ = ~0.1 S m^{-1}; L = ~0.01 m; HRT = 0.01 s; TI = 7.0–10.5 kWh m^{-3}; E = 19.7 kV cm^{-1}	PEF treatment solubilized approximately 10% of the total COD, increasing SCOD from as low as 20 mg L^{-1} to more than 1000 mg L^{-1}. VSS were disrupted into smaller coloids. AD resulted in increased BMP by 80% for pig manure, and 100% for WAS after 25 to 30 days.	Salerno et al., 2009
Excess sludge obtained from a sewage treatment factory	Electrode plates from Cu foil (50 mm in diameter and 0.1 mm in thickness); E = 5.88 to 14.7 kV cm^{-1}; τ = ~45 µs; T = 20–60 °C; f = 0.1–100 Hz	Increase of E (5.88 to 11.76 kV cm^{-1}) affected the pretreatment efficiency (change of ΔS from 6.22 to 30.11 mg L^{-1}). Increase of T resulted in increase in pretreatment efficiency. With the T increasing from 20 °C to 40 °C, ΔS is changed from 3.93 to 41.9 mg L^{-1}. As the T rises up to 60 °C, the increment is nearly 3 times larger than above. With the increase of the f, the pretreatment efficiency tends to decrease. The ΔS at 1 Hz has been found nearly twice that at 100 Hz.	Gao et al., 2015
WAS taken from the secondary clarifier at a municipal wastewater treatment plant	Co-linear cylindrical treatment chamber with stainless steel electrodes; σ = 4.32, 6.3 and 8.86 mS cm^{-1}; V = 9–54 kV; f = 50 Hz; electrode gap = 23 and 28.5 mm	AD of WAS pretreated with PEF increased the CH_4 production by 1.36 fold.	Kuşçu and Çömlekçi, 2015
Raw sludge mixture (60% surplus sludge + 40% PS) from the wastewater treatment plant	Co-linear electrode system; E = 8 to 30 kV cm^{-1}; σ = 1.6 mS cm^{-1}; $W_{spec.}$ = 150–280 kJ L^{-1}	Degree of desintegration after PEF pretreatment: up to 20%. The microscopic picture: disintegration of the sludge composition can be seen. PEF reduced foaming of sludge during AD. Biogas yield improved by up to 20% if compared to control group.	Kopplow et al., 2004
PS collected from the primary clarifier underflow at the wastewater reclamation plant	σ = 0.175 mS cm^{-1}; V = 30 kV; TI = 33 kWh m^{-3}	PEF treatment enhanced conversion of PS COD to CH_4 (by ~8%) and to VFAs by AD (by ~7%) by increasing hydrolysis rates.	Ki et al., 2015
Mixture of PS and WAS from wastewater reclamation plant	TI = 30.0–35.8 kWh m^{-3}	PEF treatment increased the CH_4 production rate and COD removal efficiency by up to 33% and 18%, respectively, at a SRT of 20 days.	Lee and Rittmann, 2011
Activated sludge from wastewater treatment plant	V = 20 kV; E = 7–19 kV cm^{-1}; f = 20–80 Hz; $W_{spec.}$ = 70–250 kJ kg^{-1}	The inner floc size structure was not effected by the treatment, but at the edges the structure appears to be disturbed and the amount of particle in the liquid increased. Circularity of the treated samples increases (PEF treatment acts upon the peripheral rim of the substrates' surface) but the diameter remains stable. PEF treatment does not affect the compact cell structure, but cause membrane perforations which reduce the resistibility of the cell.	Koners et al., 2006
Two types of WAS collected from the tickeners of WAS at two wastewater treatment plants	stainless steel reactor with a coaxial electrode and multiple ring electrodes; V = 20 kV; f = 150 Hz	Batch AD of PEF treated WAS showed 2.5 times higher biogas production than that of untreated WAS.	Choi et al., 2006
Landfill leachate and fruit/vegetable slurry	Stainless steel electrodes 20 cm long, 0.5 cm wide and 7 cm high. $d_{electrode}$ = 1 cm; f = 1.7 Hz; τ = 4 µs; E = 20 kV cm^{-1}; TI = 15, 30 and 50 kWh m^{-3}	CH_4 production of the landfill leachate significantly increased up to 44% with the highest TI (50 kWh m^{-3}). PEF pretreatment of fruit/vegetable slurry showed that 15 kW h m^{-3} was the intensity by which the highest amount of CH_4 (up to 7%) was achieved. Beyond this intensity, the CH_4 production decreased. COD removals were increased up to 100% for landfill leachate and 17% for fruit/vegetable slurry, compared to the untreated slurries.	Safavi and Unnthorsson, 2017

* V - applied voltage; f - pulse frequency; σ - sample conductivity; L - length of the treatment chamber; HRT - residence time in the treatment chamber; TI - treatment intensity; E - electric field strength; τ- pulse width; T – temperature; ΔS - difference between SCOD after and before the pretreatment; $W_{spec.}$ - energy input.

ity and other operating and maintenance costs when the biogas increase and biosolids decrease are 60% and 40%, respectively. The energy balance calculation showed the energy benefit from increased biogas production plus heat recovery about 18 times greater than the energy input for PEF pretreatment. It was concluded that the capital and installation costs for the equipment would pay back in less than three years.

Another commercial deployment of PEF technology has multiple full-scale installations in Europe and pilot plants in USA (Long and Bullard, 2014). This technology is composed of pipes with electrodes alongside that apply a voltage of around 30 to 100 kV (Trzcinski, 2019). It could be installed prior to entry into the digesting tower, treatment of sludge as part of digester recirculation or disintegration of activated sludge. A full-scale case study with this PEF treatment unit was performed at wastewater treatment plant in the USA where thickened WAS is codigested with raw PS in four anaerobic digesters. WAS is prethickened in gravity thickeners before final thickening via gravity belt thickeners. The unit was installed on the effluent of the gravity belt thickeners. During this study the primary and secondary digesters had an average 31- and 50.4-day solid retention time (SRT), respectively. A study showed increase in biogas production with high TSS and VSS disintegration (13.6 and 11.4%, respectively) and low COD solubilisation (0.30%). The process increased the rate of AD but not the extent of digestion therefore authors concluded that it may be more appropriate for plants operating with lower digester SRT (15 to 20 days) where an increase in the rate of AD would have a more impact on total biogas production (Long and Bullard, 2014).

4.2. PEF pretreatment of LC for biogas production

LC biomass from terrestrial plants, energy crops, agricultural residues and wastes is already widely used for biogas production and, as being abundant and potentially carbon–neutral source for bioenergy production, it offers a promising alternative to satisfy future energy demand (Golberg et al., 2016; Ahmed et al., 2019). The main components of LC biomass are polymers polysaccharides cellulose, hemicellulose and lignin which are organized in complex non-uniform three-dimensional structure with different degrees and varying relative composition that make its structure resistant to degradation (Tišma et al., 2018; Ahmed et al., 2019; Karrupiah and Azariah, 2019; Kovačić et al., 2019). This lignin-polysaccharides matrix limits cell walls enzymatic hydrolysis making it recalcitrant to biodegradation (Li et al., 2016). Therefore, a proper pretreatment is an essential step in LC biomass conversion to biogas. The pretreatment is focused on improving the cellulose and hemicellulose accessibility and should result in lignin removal, reduction of cellulose crystallinity and an increase of the material porosity, and preservation of hemicelluloses. Efficient pretreatment is defined by the enhanced formation of sugars in further enzymatic step of biomass processing and by inhibitory compounds formation that is limited to minimum (Kucharska et al., 2018).

The existing methods in LC biomass conversion to biogas include mechanical, physical, chemical, and biological pretreatments which were in detail discussed in our previously published review paper (Kovačić et al., 2017). However, some of these methods may require a long processing time (e.g. biological methods), some may require large amounts of chemicals and solvents (e.g. chemical methods) and some may be energy consuming (e.g. physical methods). Though, recent applications of PEF in LC biorefinery have demonstrated their high potential. So far, several studies are found in the literature dealing with LC biomass PEF pretreatment. All of them have demonstrated a considerable potential for assistance in processing (degradation, decomposition, extraction, digestion, and dewatering) of wood and crop based LC materials (Vorobiev and Lebovka, 2017). In LC treatments PEF is mostly found as a method used for extraction of intracellular components like carotenoids, vitamins, sucrose, proteins, inulin, polyphenols and other substances (Bozinou et al., 2019; Maza et al., 2020). PEF treatment induce formation of micro and nanopores with low temperature effects which is quite important for heat-sensitive materials (Shorstkii et al., 2020) and was proven as method that can improve the extraction efficiency from LC materials by increasing the extraction yield, decreasing the processing time, and reducing the energy requirements of the process and subsequent processes (Tamborrino et al., 2020). PEF has been so far investigated by different groups for the extraction of proteins and polyphenols from plant. *E* used in those researches was in the range from 0.9 to 20 kV cm^{-1} and resulted in significant increases of extracted content. For instance, Bouras et al. (2016) achieved to extract up to 8 times higher phenolic content after application of PEF to Norway spruce bark in comparison to extraction of phenolic content from untreated sample. Zderic et al. (2013) pretreated fresh tea leaves by applying PEF (*E* in the range from 0.1 to 1.1 kV cm^{-1}) to extract polyphenols. Their results showed that extraction yield increases with increasing *E*. Maximum extraction yield (27%) was achieved after applying *E* of 0.9 kVcm^{-1} and pauses between pulses that last for 0.5 s. By increasing intervals between pulses an *E* of 1.1 kV cm^{-1} is needed to obtain the same extraction yield. Longer pulses (and accordingly longer treatment time, 2.5 s) were more effective for moderate *E* (0.4 kV cm^{-1}). To achieve the same extraction yield of 27% but with shorter treatment time (1.5 s), higher *E* (0.9 kV cm^{-1}) is required. Yu et al. (2015) pretreated rapeseed stems and leaves with PEF by applying *E* of 0.8, 5 and 20 kV cm^{-1} to extract polyphenols. PEF pretreatment in the range *E* = 5–20 kV cm^{-1} increased the extraction of total polyphenols. Treatment at *E* = 5 kV cm^{-1} resulted in the highest polyphenols purity and was chosen as the optimum *E* to recover selectively polyphenols from rapeseed stems. In another study of Yu et al. (2016), rapeseed green biomass was pretreated by applying *E* in the range from 5, 8, 10 and 20 kV cm^{-1} and number of pulses in the range from 50 to 400 to extract rapeseed juice. Juice yield increased from 34% to 81% under optimal treatment conditions (*E* = 8 kV cm^{-1}, t_{PEF} = 2 ms, *P* = 10 bar). Significant increases in total polyphenols content, total protein content and in consolidation (compression) coefficient were also observed after PEF treatment. Nowacka et al. (2019) studied the influence of the PEF (*E* = 4.38 and 6.25 kV cm^{-1}) as a pretreatment method for the extraction of betalains from beetroot. The obtained results showed that the application of PEF pretreatment significantly influenced the efficiency of extraction of bioactive compounds from beetroot. The highest increase in the content of betalain compounds in the red beet's extract (betanin by 329% and vulgaxanthin by 244%, compared to the untreated sample) was noted at *E* = 4.38 kV cm^{-1}, 20 impulses and energy consumption of 4.10 kJ kg^{-1}. Gachovska et al. (2009) employed PEF (*E* = 1.25, 1.90, and 2.50 kV cm^{-1}; variations in pulse numbers and capacitance of the discharge capacitor) to determine the relationship between spent energy and the maximum damage degree after extraction of juice from alfalfa mash. In the rate of damage beyond 0.5 kJ of applied energy no significant increase was observed. The highest rate of change of the damage degree at 0.5 kJ was achieved when the capacitance was 1.5 μF for all the voltages. Increase in the *E* resulted with decrease in energy which is needed to achieve the maximum damage degree. The capacitance of the discharge capacitor should rather be 1 μF or more so that an efficient result for alfalfa juice extraction could be achieved. In the conclusion authors suggested fewer number of pulses and the highest energy per pulse to minimize energy consumption for a given damage degree in alfalfa.

Other authors studied the effect of PEF on tissue degradation and decomposition and they tested *E* in the range from 0.5 to 15 kV cm^{-1}. All treatments resulted in significant membrane destruction and porosion. For instance, Kumar et al. (2011) pretreated wood chips and switchgrass by applying PEF of *E* in the range of 1 to 10 kV cm^{-1}, respectively, and studied the uptake of dye in untreated and treated samples to quantify the effect of PEF treatment on diffusion in tissues. The switchgrass samples were resistant to change in the structure at low *E* up to 5 kV cm^{-1}, while the samples treated at *E* of $\geq$ 8 kV cm^{-1} showed faster and larger dye uptake suggesting an increase in the porosity of treated material. Similar phenomena were observed for wood chip samples treated at 10 kV cm^{-1}. Therefore, authors suggest PEF pretreatment as a method that can increase enzymatic reaction rate or acid hydrolysis of biomass samples in the production of biofuels. Han et al. (2020) applied PEF treatment for preparation of porous corn starch which is largely used in chemical, pharmaceutical, and agri-food industry for transportation or protection of pigments, spices, and sensitive substances like vitamins, functional fatty acid, and anthocyanin. Authors firstly used enzymes to hydrolyze corn starch and afterwards they applied *E* in the range from 9 to 15 kV cm^{-1}. The impact of treatments was investigated by oil absorption to porous starch which was highest after 3.9 hr of enzyme catalized reaction, followed by PEF treatment at 11.5 kV cm^{-1} for 18 ms. Kim et al. (2019) investigated effect of PEF (*E* = 0.5, 1.5, and 2.5 kV cm^{-1}) on physicochemical properties of raw ginseng root. Ginseng cell and vacuole membranes were significantly affected by PEF treatment at field strengths greater than 1.5 kV cm^{-1}. Increased cell membrane disintegration accompanied with increased ion leaching after PEF treatment are evidences of changes in membrane systems. Subsequent observation

Table 4
Operational settings of lab- and pilot-scale studies of PEF pretreatment of LC for different purposes.

Feedstock	PEF pretreatment parameters	Application	Effects of the pretreatment	Reference
Wood chips and switchgrass	Wood chips (particle size ~ 1.5 mm, suspended in distilled water): n = 1000 and 2000 pulses, E = 1 kVcm^{-1}; and n = 1000, 2000 and 5000 pulses, E = 10 kV cm^{-1}; τ = 100 µs; f = 3 Hz. Switchgrass (particle size between 0.85 and 1.65 mm, suspended in distilled water): n = 1000, 2000 and 5000 pulses, E = 2.5, 5, 8 and 10 kV cm^{-1}; τ = 100 µs; f = 3 Hz.	To study the effect of electroporation on the pore formation in LC and to determine the extent of permeabilization of the biomass after pretreatment.	The samples treated at E = ≥8 kV cm^{-1} showed faster and larger neutral red dye uptake, suggesting an increase in the porosity of switchgrass samples.	Kumar et al., 2011
Rapeseed green biomass (stems)	Pulse generator of 40 kV and 10 kA. Treatment chamber with two parallel disks stainless electrodes. Substrate mixed with distilled water. E = 5, 8, 10 and 20 kV cm^{-1}; n = 50, 100, 200 and 400 pulses; D = 10 µs; f = 0.5 Hz; Δt = 2 s.	To study the effects of PEF pretreatment on the valorization of extractives (proteins and polyphenols) from rapeseed green biomass obtained by pressing.	After applying E = 8 kV cm^{-1}, t_{PEF} = 2 ms and P = 10 bar the expressed juice yield increased from 34% to 81%. Significant increases in total polyphenols and total proteins content and in consolidation coefficient were observed after PEF pretreatment. The dry matter of recovered press cake increased from 8.8% to 53.0%.	Yu et al., 2016
Norway spruce bark	Pulse generator of 40 kV and 10 kA. Particle size of the substrate = 1.2 mm. Cylindrical treatment chamber with stainless steel disk electrodes. PEF generator of 40 kV and 10 kA. E = 20 kV cm^{-1}; $d_{\text{electrodes}}$ = 2 cm; f = 0.5 Hz; n = 25, 50, 100, 200, 300 and 400 pulses; D = ~10 ± 1 µs.	To improve aqueous solid/liquid extraction of the polyphenols contained in Norway spruce bark by the use of PEF pretreatment.	The PEF treatment enhanced extraction of total phenolic content and antioxidant activity. The total phenols content has increased of more than 8 times after PEF treatment. The analysis showed that PEF treatment does not affect the structure of wood.	Bouras et al., 2016
Fresh tea leaves	σ = 3.5 mS cm^{-1}; $d_{electrodes}$ = 4 cm; E = 0.1–1.1 kV cm^{-1}; D = 0.0001–0.1 s; n = 10–50 pulses; Δt = 0.5, 3 and 5 s.	To study the effect of PEF pretreatment and total treatment time on the extraction yield of polyphenols.	The extraction yield increased with increasing E. After applying E = 0.9 kV cm^{-1}, n = 30 pulses, D = 0.05 s and Δt = 0.5 s maximum value of extraction yield (27%) was achieved. Longer pulses are more effective and their effect is particularly pronounced at a moderate electric field, E = 0.4 kV cm^{-1}.	Zderic et al., 2013
Rapeseed hulls	Pulse generator of 40 kV and 10 kA. Treatment chamber equipped with two parallel disc electrodes. Substrate mixed with distilled water. D = 10 µs; f = 0.5 Hz; $d_{\text{electrodes}}$ = 3 cm; E = 13 kV cm^{-1}; $W_{spec.}$ = 150 kJ kg^{-1}; n = 599 pulses; τ = 10 µs.	PEF pretreatment was applied prior to chemical treatment of rapeseed hulls. PEF pretreatment was applied prior to chemical and enzymatic treatment of rapeseed hulls with the aim to enhance the removal of acid insoluble residue from rapeseed hulls.	Applying PEF pretreatment resulted in an increasing yield of acid insoluble residue by 5%.	Brahim et al., 2016
Alfalfa stems and leaves	Plastic insulated cylinder housed in treatment chamber. σ = 3.5 mS cm^{-1}; f = 1 Hz; E = 1.25, 1.9 and 2.5 kV cm^{-1}; C = 0.5, 1 and 1.5 µF.	To study the influence of PEF parameters on the damage degree of alfalfa mash, and to determine the relationship between the maximum damage degree and the energy used.	There was no significant increase in the rate of damage beyond 0.5 kJ applied energy. The rate of change of the damage degree at 0.5 kJ was highest when the capacitance was 1.5 µF for all the voltages. Increase in the E led to decrease in energy needed to obtain the maximum damage degree. To achieve an efficient result for alfalfa juice extraction, the C of the discharge capacitor should be 1 µF or more.	Gachovska et al., 2009
Rapeseed stems and leaves	Substrate mixed with water (solid to liquid ratio 1:4). Moderate PEF generator of 5 kV and 1 kA. E = 0.8 kV cm^{-1}; n = 100 pulses; τ = 0.1 ms; D = 1 ms; Δt = 100 ms; N = 20; D = 0.1 ms; f = 0.5 kHz; t_{PEF} = 200 ms. Δt_t = 10 s. High electric field generator of 40 kV and 10 kA. E = 5 and 20 kV cm^{-1}; n = 200 pulses; τ = 10 µs; N = 1; Δt_t = 2 s; f = 0.5 kHz; t_{PEF} = 2 ms.	To study the effect of PEF on the extraction of polyphenols and proteins from rapeseed stems and leaves.	The PEF (E = 5–20 kV cm^{-1}) increased the extraction of total polyphenols from rapeseed stems and leaves. Treatment at E = 5 kV cm^{-1} resulted in the highest polyphenols purity. The polyphenols content in rapeseed stems and leaves decreased (≈74%) as plant maturity advanced while the change for protein content is more gentle (≈ 35.6% decrease).	Yu et al., 2015
Native corn starch	σ = 150 µs cm^{-1}; f = 1000 Hz; D = 40 µs; t_{PEF} = 10.8, 14.4 and 18.0 ms; E = 9–15 kV cm^{-1}.	PEF pretreatment was applied together with enzymatic treatment to prepare porous corn starch and to study its physicochemical characteristics after pretreatment.	The highest oil absorption value (130%) was achieved after applying it to corn starch wich was subjected to enzymolysis for 3.9 hr, and was pretreated with PEF under E = 11.5 kV cm^{-1} and t_{PEF} = 18 ms.	Han et al., 2020
Raw *Panax ginseng* roots	Pulse generator of 5 kW. Substrate mixed with tap water. The batch treatment chamber with parallel electrodes. E = 0.5	To study the effect of PEF on physicochemical properties of raw *Panax ginseng* roots.	Ginseng cell and vacuole membranes were significantly affected by PEF treatment at E = >1.5 kV cm^{-1} and were	Kim et al., 2019

(*continued on next page*)

Table 4 (*continued*)

Feedstock	PEF pretreatment parameters	Application	Effects of the pretreatment	Reference
	to 2.5 kV cm^{-1}; n = 500 pulses; f = 50 Hz; τ = 25 µs.		verified by increased ion leaching and increased cell membrane disintegration. Texture profile analysis showed a significant decrease in hardness (by 44%) and chewiness (by 45%) at E = 2.5 kV cm^{-1}. Total phenolic content and antioxidant activity were significantly increased by 11% and 3.64 to 21.4%, respectively.	
Beetroot (*Beta vulgaris* L.)	Substrate mixed with phosphate buffer at pH = 6.5. The treatment chamber made of dielectric material equipped with two stainless-steel lids and set of electrodes. τ = 10 µs; Δt = 2 s; E = 4.38 and 6.25 kV cm^{-1}; n = 10, 20 and 30 pulses; $W_{spec.}$ = 2.43–14.88 kJ kg^{-1}.	To study the influence of the PEF as a pretreatment method in the extraction of betalains from beetroot.	PEF pretreatment significantly influenced the efficiency of extraction of betalains from beetroot. The highest increase in the content of betalains in the red beet's extract (betanin by 329%, vulgaxanthin by 244%, compared to the control sample), was noted for n = 20 pulses and E = 4.38 kV cm^{-1}. Increase in the electrical conductivity compared to the non-treated sample was also determined due to the increase in cell membrane permeability.	Nowacka et al., 2019

* E - electric field strength; n - number of pulses; f - pulse frequency; τ - pulse width; D - pulse duration; $d_{electrodes}$ - distance between the electrodes; σ - sample conductivity; t_{PEF} - PEF treatment specific time; P – pressure; C - capacitance of the discharge capacitor; Δt - time duration between pulses; Δt_t = time duration between series of pulses; N - series of pulses; t_{PEF} - total pretreatment time; $W_{spec.}$ - energy input.

using electron microscopy vacuole destruction and plasma membrane separation were revealed after PEF treatment. Texture profile analysis showed a significant decrease in hardness (by 44%) and chewiness (by 45%) at E = 2.5 kV cm^{-1}. Total phenolic content (by 11%) and antioxidant activity (up to 21.4%) were significantly increased.

Brahim et al. (2016) applied different pretreatment methods (ultrasound, microwaves, high voltage electrical discharges and PEF) before alkaline pretreatment in order to evaluate their effects on acid insoluble residue removal and enzymatic hydrolysis yields from rapeseed hulls. The results showed increased yield of acid insoluble residue by 5% after PEF pretreatment of hulls compared to the untreated sample. If compared with the results of other pretreatments (ultrasound increased yield by 12%, microwave by 6%, high voltage electrical discharges by 8%), PEF pretreatment resulted in lowest enhancement in acid insoluble residue yield.

All PEF operational settings and parameters of above mentioned studies are presented in Table 4 in detail.

Although PEF has been applied to different tissues and for different purposes, applications to LC for enhancement of biogas production are scarce. However, all of performed studies, which have been carried out on laboratory scale, resulted in enhancement of biogas and/or CH_4 production and positive energy balance (where calculated). Hence, in view of future industrial exploitation of this pretreatment method in biogas production, further investigations and additional developments at lab-scale are needed, as well as their evaluation from economic and environmental point of view (Golberg et al., 2016). Only a few studies have investigated the production of biogas from LC which was previously pretreated by PEF. Lindmark et al. (2014) presented results performed to evaluate the potential of PEF technology for enhancing biogas production from ley crop silage. Different settings of the PEF equipment were tested, and the results of pretreatment were tested using a batch AD setup. After AD of pretreated lay crop silage CH_4 yield (under E = 96 kV cm^{-1}; n = 65 pulses; f = 5 Hz; σ = 6.80 mS cm^{-1}) increased by 16% after 365 days of digestion in comparison to untreated substrate. Pretreatment of lay crop silage thus reduced digestion time by 30% compared to untreated substrate. The energy balance shows that the pretreatment can produce up to a 2-fold larger energy output in the form of CH_4 compared to the electrical energy input. There was no significant difference between samples treated at lower field strength (48 kV cm^{-1}) and untreated sample. El Achkar et al. (2018) compared the effects of pretreatment of different pretreatment methods (freezing, alkaline treatment using NaOH and NH_3, acid treatment using HCl, ultrasounds and PEF) on biodegradability of LC components of grape pomace and AD of grape pomace. Afterwards they validated the most efficient pretreatment (in terms of CH_4 production) on larger scale continuous digesters. Although PEF pretreatment was not the one that gave best results after AD, the 9 trains level of PEF ($W_{spec.}$ = 41 kJ kg^{-1}) resulted in increase of the cumulative CH_4 yield and the hydrolysis constant by 1% and 9%, respectively, while the 14 trains level of PEF ($W_{spec.}$ = 153 kJ kg^{-1}) resulted in increase of the cumulative CH_4 yield and the hydrolysis constant by 4% and 14%, respectively. After determination of LC composition, authors revealed that PEF pretreatment did not affect the chemical composition of grape pomace. They concluded that PEF probably induced appearance of pores in cell membranes releasing some components to the extracellular medium which made grape pomace more amenable to hydrolysis that resulted in improved AD process. Wang et al. (2018) pretreated the aboveground part of hybrid Pennisetum in different conditions of high voltage pulsed electric field (HPEF) to improve its material utilization ratios and rate of biogas production. Nine groups of hybrid Pennisetum pretreated by HPEF were superior in biogas production efficiency, cumulative biogas production, peak daily biogas production and in maximum CH_4 concentration, in comparison to control group. The highest enhancement in biogas production occurred after HPEF treatment with 15 kV, 120 Hz during 60 min where cumulative biogas production resulted in 27% higher yield than that of the control group, the peak daily biogas production increased and the range of peak period extended. Kovačić et al. (2019) applied PEF (E_{min} = 0.897 k V cm^{-1}, E_{max} = 13.90 kV cm^{-1}) for pretreatment of different harvest residues (corn stover, soybean straw and sunflower stalk) in order to enhance biogas and CH_4 yield. Compared to untreated harvest residues biogas and CH_4 yields increased by 18 and 16%, respectively, after pretreatment (E = 0.935–1.664 kV cm^{-1}, t_{PEF} = 10 min) of corn stalks; 18 and 17%, respectively, after pretreatment (E = 0. 760–1.354 kV cm^{-1}, t_{PEF} = 30 min) of soybean straw. There was no statistically significant difference between pretreated and untreated sunflower stalks. After anaerobic co-digestion of pretreated harvest residues, energy balance was calculated for total processes. The highest positive energy balance with energy gain of + 13.30 kWh t^{-1} was achieved after pretreatment (E = 0.935–1.664 kV cm^{-1}, t_{PEF} = 30 min) of corn stover; energy gain of + 14.95 kWh t^{-1} after pretreatment (E = 5.714–6.108 kV cm^{-1}, t_{PEF} = 20 s) of soybean straw; and energy gain of + 15.25 kWh t^{-1} after pretreatment (E = 0.897–1.596 kV cm^{-1}, t_{PEF} = 30 min) of sunflower stalk. Recently, PEF has also been studied as pretreatment method for the production of biogas from algae. Garoma and Shackelford (2014) pretreated algae *Chlorella vulgaris* by applying PEF of *TI* in the range from 2 to 150 kWh m^{-3} in order to enhance biogas production. The effectiveness of the *TI* was measured using increase in SCOD. The optimal *TI* occurred at 28 kWh m^{-3} under which solubilisation of SCOD was significantly improved to more than 830%. The enhancement in biogas production after pretreatment at 5.4 kWh m^{-3} was around 27%. Net volume of CH_4 production was significantly higher for all pretreatments compared to untreated sample.

At 35 kWh m^{-3} CH_4 production increased by as high as 110%. Authors also pointed out that lower *TI* levels resulted in high rates of gain per energy input.

All PEF operational settings and parameters of above mentioned studies are summarized in Table 5.

Table 5
Operational settings and parameters of lab- and pilot-scale studies of PEF pretreatment of LC for biogas production.

Feedstock	PEF pretreatment parameters	AD parameters	Effects of the pretreatment	Reference
Ley crop silage	Pulse generator of 40 kV and 40 mA. Vertical electrode configuration; f = 5 and 10 Hz; $d_{electrodes}$ = 0.05 and 0.025 m; E = 48 and 96 kV cm^{-1}; n = 65 and 100 pulses; σ = 5.80–7.00 mS cm^{-1}.	Batch-scale anaerobic digesters operating in mesophilic regime (30 °C) without mixing; pretreated ley crop silage inoculated with active anaerobic sludge of 0.9 g VS; inoculum to substrate ratio of 0.9.	The biogas yield was increased by 16% by applying n = 65 pulses, E = 96 kV cm^{-1}, and *Wspec.* = 259 Wh kg^{-1} VS. However, at n = 100 pulses, E = 48 kV cm^{-1} and the same total energy input (*Wspec.* = 259 Wh kg^{-1} VS), no effects of the pretreatment were observed. The energy balance of the PEF pretreatment shows that the CH_4 yield can be up to double the electrical energy input of the process.	LindmLindmark et al., 2014ark et al., 2014
Grape pomace	Pulse generator of 2.5 kV and 0.24 A. A treatment chamber made of plexiglass with two steel electrodes (inner diameter of 6.5 cm). $\tau = 10^{-5} - 10^{-4}$ s; f = 24–240 Hz; t_{PEF} = 0.36 and 1 s; rectangular monopolar trains n = 240 pulses, with $\tau = 50 \cdot 10^{-6}$ s, N = 9 and 14 trains ; $W_{spec.}$ = 41 and 153 kJ kg^{-1} for 9 and 14 trains, respectively.	Batch-scale anaerobic digesters operating in mesophilic regime (37 °C) for determination of BMP. The selected pretreatment conditions were validated on a larger scale using continuous digesters (t = 70 days) at mesophilic regime (37 °C) with mixing (*HRT* = 20 days, *OLR* = 3.7 kg COD m^{-3} day^{-1}). Inoculum to substrate ratio 3:1 (based on COD).	PEF pretreatment did not affect the chemical composition of grape pomace. Application of W = 153 kJ kg^{-1}, t_{PEF} = 1, N = 14 s increases the cumulative CH_4 yield and the hydrolysis constant by 4% and 14%, respectively.	El Achkar et al., 2018
Harvest residues (soybean straw, corn stalk and sunflower stalk)	Cu electrodes; $d_{electrode}$ = 2.03–2.99 mm; U = 200–365 V, 1.16–1.24 kV, 3.00–3.10 kV; E_{min} = 0.897 kV cm^{-1}; E_{max} = 13.90 kV cm^{-1}; $t_{PEF,\ min}$ = 20 s, $t_{PEF,\ max}$ = 40 min.	Batch-scale anaerobic digesters operating in termophilic regime (55 °C) wit manual mixing (t = 26 days); pretreated harvest residues codigested with dairy cow manure (ratio 1:2.4 on the TS basis).	After applying PEF pretreatment, increase of up to 18% in biogas yield (by applying U = 200–365 V, E = 0.935–1.664 kV cm^{-1}, t_{PEF} = 10 min) and 17% in CH_4 yield (by applying U = 200–365 V, E = 0.760–1.354 kV cm^{-1}, t_{PEF} = 30 min) can be achieved after AD. A positive energy balance of up to E_{SUM} = 15.25 kWh t^{-1} was achieved after PEF pretreatment and subsequent AD.	Kovačić et al., 2019
The aboveground part of hybrid Pennisetum	V = 15 kV, 26 and 40 kV; τ_{max} = 500 ns; f = 90, 120 and 150 Hz; t_{HPEF} = 30, 60 and 90 min (parameters were combined).	Batch-scale anaerobic digesters operating in mesophilic regime (35 ± 1 °C) during 32 days; pretreated Pennisetum, sludge and warm water mixed in ratio 1:100:200.	After applying HPEF pretreatment (V = 15 kV, f = 120 Hz, and t_{HPEF} = 60 min) and subsequent AD, the biogas yield increased by 26.9%.	Wang et al., 2018
Algae Chlorella vulgaris	Pulse generator of 3 kV and 40 mA. TI = 2–150 kWh m^{-3}; τ = 5, 10 and 20 µs; V_{max} = 2.4 kV.	Batch-scale anaerobic digesters operating in mesophilic regime (35 °C) and 150 rpm; pretreated algal biomass inoculated with seed bacteria; substrate to inoclum ratio 1:1 on the basis of VS.	Pretreating algal biomass with PEF significantly improved the SCOD, increasing it to more than 830% at TI = 28 kWh m^{-3}. Culture conditions also affected the performance of the PEF process. On the basis of SCOD, a sample pH = 7.0 and cell concentration of 13.2 g L^{-1} were found to be optimal for the PEF pretreatment. At TI = 35 kWh m^{-3}, CH_4 production increased by as high as 110%. Lower *TI* levels resulted in high rates of gain per *Wspec.* compared to higher degrees of treatment.	Garoma and Shackelford, 2014

* *TI* - treatment intensity; τ- pulse width; V_{max} - maximum voltage applied; f – frequency; $d_{electrodes}$ - distance between the electrodes; n - number of pulses; σ - sample conductivity; t_{PEF} - PEF treatment specific time; W_{spec} - energy input; E_{min} – minimum electric field strength; E_{max} – maximum electric field strength; t - duration of digestion; *HRT* - hydraulic retention time; *OLR* - organic loading rate; N - number of trains; t_{HPEF} – high-voltage pulsed electric field treatment time; *HPEF* - high-voltage pulsed electric field.

5. Future perspectives

It is certain that this method will evolve in the upcoming years as its potential is beyond question. However, the following obstacles and issues need to be prevailed:

So far, there are no established procedures and principles according to which this method could be implemented in biogas industry because of numerous parameters that can be adjusted during pretreatment as well as very different implementations of equipment. Therefore, it is of great importance to conduct extensive research and define optimal parameters and procedures for particular types of substrates to make it reproducible. Economic and energetic evaluation of the whole process should be calculated so that the cost-effectiveness of the process could be evaluated.

Application of PEF technology in biogas production from wastewaters and sludges is well-established and continuous industrial scale units already exist. However, biogas production from solid LC substrates is in its early research phase in which laboratory-scale studies are conducted using small sample volumes. A benefit and current knowledge from food industry and wastewaters processing can be derived for PEF treated LC-derived biogas production. What is important to be discussed in regard to further development related to equipment and processing design is specified further in the text.

After extensive lab- and pilot-scale research, it is necessary to scale up the process in order to evaluate the feasibility of the method in large industrial scale and its safety because scale up requires a far greater power regime which creates unsafe working conditions and limits cost-effectiveness of the process. High initial investment costs represent one of the major concerns in commercialization of PEF technology.

The fundamental distinction between lab- and pilot-scale, and industrial scale PEF treatment units is average power which correspondence to throughput capacity. While most lab-scale units are rated at a few kW or less average power, industrial scale units are rated around 30 kW. Pilot-scale units can operate between these regimes and sometimes can be used for small commercial operations. Therefore, the power supply must be strong enough to provide the power supply capacity required for industrial-scale units.

The main issue related to up-scaling of LC PEF treatment is related to treatment chamber design and its volume. A possible solution could be transferred from PEF treatment of pumpable products. The idea is to use parallel systems with several treatment chambers which have small gaps between electrodes in line rather than trying to build large volume systems because in the case when more systems are working together in a production line and one of them fails, other systems would continue working and remain operational (Mastwijk and Bartels, 2001; Galindo and Henriksson, 2012). The non-uniformity of the electrical field distribution that exists in treatment chambers with small gaps between electrodes perhaps could be prevailed after passing through multiple treatment chambers in line which could result with a uniformly treated substrate.

On the other hand, in treatment chambers with larger gaps between electrodes a temperature gradient appears across the treatment zone. According to Gavahian et al. (2020), a slight temperature increase (result of Ohmic heating) affects the increase in permeability of the cell tissue and result in the leakage of cell ingredients. Leakage of cell ingredients can in turn increase the electrical conductivity of the extracted medium and enhance the heating rate which can positively affect the process in the way that more intracellular components become more accessible for subsequent anaerobic hydrolysis. However, in the case when the higher temperature increment that occurs after passing through each treatment chamber is undesirable, it could be removed by passing through cooling zone which should be installed between treatment chambers.

In biogas industry PEF pretreatment method is restricted to liquids (wastewaters and sludges) while pretreatment of solid substrates (e.g. LC) is only in its initial phase of research and is limited to research and development level. The most important limitation of solid substrates is lack of fluidity and non-uniformity of treatment. Therefore, the above mentioned technique for LC pretreatment may be applicable after adding sufficient water to substrate to create a pumpable mash.

6. Conclusion

Application of PEF in biogas production from LC biomass represents a challenge and very attractive approach since it holds great promises for biogas production. LC pretreatment by PEF method is only in its infancy, and currently is in research phase in which researchers are investigating whether there is and to which extent, influence of the PEF on enhancement of the biogas production process and most attention is paid to the pretreatment process parameters and not to the equipment and its performances.

This review has risen up many questions in need of continuation of further investigation. It is recommended to gather an interdisciplinary team in further research because this method is very specific and requires collaboration of several professions for such specific equipment handling and maintenance, better understanding of the processes that occur within a cell during electric field exposure, and experts in the field of AD.

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Submission declaration and verification

This work has not been published previously, it is not under consideration for publication elsewhere, its publication is approved by all authors and tacitly or explicitly by the responsible authorities where the work was carried out, and that, if accepted, it will not be published elsewhere in the same form, in English or in any other language, including electronically without the written consent of the copyright-holder.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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