



Pulse Electric Field Technology for Wastewater and Biomass Residues' Improved Valorization

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Review

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Abstract: Development and adoption of more efficient and robust technologies for reuse of wastewater embedded resources, in particular materials and energy, is becoming an unavoidable necessity. Among many emerging technologies in the sector of wastewater treatment residuals valorization, Pulsed Electric Field (PEF) processes have shown interesting potential, although they have not yet entered the sector's mainstream as a consolidated commercial technology, as in other industrial applications, such as the food, medical, and bio-based industries. PEF is a non-thermal technology suitable to biological applications, involving gentle cell disintegration and enhanced cell membrane permeability and as such applicable to disinfection, sterilization, and to those processes that benefit from an enhanced extraction of organic compounds from biological matter, such as anaerobic digestion, biological processes for recovery of nutrients, and biorefinery of cell-embedded compounds. PEF technology applications in wastewater/biomass residues management are reported and advantages, drawbacks, and barriers of the technology are discussed in this paper.

Keywords: pulse electric field; wastewater; wastewater sludge; energy; materials recovery; circular economy; sustainable technology

1. Introduction

Increasing pressure towards the adoption of sustainable, Circular Economy schemes in all sectors of modern society, is prompting development and adoption of more efficient and robust technologies for reuse of wastewater embedded resources, in particular materials and energy [1,2]. Among many emerging technologies in the sector of wastewater treatment, in particular for the valorization of wastewater treatment residuals including sewage sludge, Pulsed Electric Field (PEF) processes have recently shown interesting potential, although they have not yet entered the mainstream in this sector as a consolidated commercial technology.

PEF processing is a growing electro-magnetic technology already employed in medical [3], food [4], and bio-based [5] industries. PEF technology has received increasing attention in past decades for the extraction or insertion of molecules and proteins into cell membranes, to achieve nonselective increase of drugs or genetic material in cells, to fuse cells with tissues, to induce intracellular effects such as the release of calcium, and modify texture and physical properties of plant tissues [6]. PEF is based on the application of high intensity (>0.1 kV/cm) and short duration (micro- to nano- seconds) electric fields to a biological matrix positioned between two electrodes, leading to electroporation (sometimes called electropermeabilization) of cell membranes. Electroporation is defined as the modification of cell membranes' permeability through the generation of nanometer-size pores, after exposing them to a strong electric field. Electroporation leads to increase in their permeability to ions, water, and other molecules.

Among the many current applications, PEF is recognized as a promising non-thermal technology for food preservation, since it allows maintaining foodstuff properties while inactivating vegetative bacteria and yeasts. Its use for pasteurizing milk, juices, or other



Citation: Capodaglio, A.G. Pulse Electric Field Technology for Wastewater and Biomass Residues' Improved Valorization. *Processes* **2021**, *9*, 736. https://doi.org/10.3390/ pr9050736

Academic Editor: Jorge A. Ferreira

Received: 7 April 2021 Accepted: 19 April 2021 Published: 22 April 2021

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Copyright: © 2021 by the author. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https:// creativecommons.org/licenses/by/ 4.0/). liquid food products is now common, while other applications, particularly those related to its potential for mass transfer improvement (e.g., increasing of the yield of vegetable oils and polyphenols extraction, accelerating drying rates or improve industrial processing of fried vegetables), are being implemented in solid foods processing [4]. Such developments and improved understanding of the mechanisms and impact of this processing technology on biological cells suggest that PEF could become an important tool for improvement of energy-efficient biosolids exploitation technologies.

The PEF process is based on the creation of a potential difference across a conductive biological material placed between two electrodes; this creates an electric field which intensity depends on applied voltage, shape of electrodes, and distance between them. Electroporation occurs due to the thus induced transmembrane potential [7]. In conventional PEF processing the electric field is applied in intervals in the range of micro- to milliseconds; in nanosecond PEF (nsPEF) processing, much higher electric fields (10–100 kV/cm) are applied for intervals of 1–300 ns, inducing distinct intracellular effects from those of conventional PEF [8]. In both cases, the resulting electroporation increases mass transfer of cell-bound molecules and ions, giving rise to promising applications in diverse fields of biotechnology (e.g., extraction of cellular compounds, stimulation of cell growth, and microbiological inactivation). The size and persistence of pores created by electric pulses depends on pulse duration: short (e.g., $100 \ \mu s$) duration pulses create smaller pores than longer (e.g., 2 ms) pulses. For practical applications, it is important to determine not only the size and number of pores created by pulses, but also how long they will remain open. It could be expected that larger pores would need more time to reseal, as experiments on mouse plasma cells showed that the original impermeability of the membrane subject to 100 μ s pulses was restored after about 6–7 min, while resealing was slower (15–20 min) when the same cells were exposed to 2 ms pulses. Multiple repeated short pulses increase pore number, compensating the smaller size of the pores created [9]. By adjusting process parameters, the desired degree of electroporation for a specific effect may be achieved and controlled.

PEF technology is suitable to biological applications involving gentle cell disintegration and cellular compounds extraction processes. Electroporation induced by PEF processing results in increased extraction yields of cell-derived compounds such as carbohydrates, proteins, and lipids, and water elimination without loss of cell integrity [10] by diffusion gradient-assisted release. While PEF processing permeabilizes cell membrane, it does not cause its complete disruption, as in other processes (e.g., microwaves); this limits the extraction of cell-bound compounds on one side, but does not impede cells growth [11], giving highly promising perspectives in future biotechnological applications. Controlled biological growth stimulation has been achieved under nsPEF processing for various organisms treated with 100 ns pulses at electric fields of 10 kV/cm under strict process characterization and control [12]: photoautotrophic *Arthrospira platensis* and *Chlorella vulgaris* and heterotrophic *Saccharomyces cerevisiae* showed increase in biomass concentrations between 13 and 20% under those conditions [13].

On the other hand, if the electric field is strong enough, and pulse duration is long enough, cell membranes can be irreversibly damaged, and the exposed organisms will die. For example, PEF technology was applied to the disinfection of combined sewer overflow (CSO) discharges by inducing 5–9 log pathogen cells destruction with 1–10 ms pulses at 1–35 kV/cm fields [14]. In addition to disinfection and sterilization, PEF technology's specific applications in wastewater/biomass residues management have been tested to enhance conversion of organic solids into biogas via anaerobic digestion [15], enhancement of bacterial processes activity [16], and extraction of biofuel precursors [17].

This paper reviews actual and potential applications of PEF electroporation technology for the creation of improved wastewater and biomasses residuals management schemes.

2. Fundamentals of Pulsed Electric Field-Induced Electroporation

Notwithstanding the established industrial applications of PEF technology, there are still notable gaps in the understanding of the electroporation phenomenon. Current understanding is based on the dielectric breakdown theory, which considers a selective damage of biological membranes. These consist almost entirely of cholesterol and phospholipids, which are composed of polar glycerol (hydrophilic) and nonpolar hydrophobic hydrocarbon parts. Within the membrane, the latter are oriented inwards, polar heads outwards, making this structure almost impenetrable for polar molecules. Under certain conditions (i.e., high temperature and/or surface tension) water and monoatomic ions may permeate them due to the temporary formation of very small, unstable pores in the lipid layer, which may become more stable under an external electric field acting on the membrane [18].

Biological cell membranes, filled with low permittivity dielectric material, could be considered as capacitors, maintaining an electrochemical gradient on both sides due to the accumulation of negative ions at the inner surface, and the presence of an equal number of positive ions outside. This builds a transmembrane potential ("resting potential") across the membrane wall. After exposure to an external electric field of sufficient intensity, ions within the membrane migrate toward the outside, with accumulation of free charges at both sides of the wall, increasing the potential difference across the membrane. The additional transmembrane potential induced by the electric field, larger than the natural one, is unevenly distributed over the membrane surface. Upon locally reaching a critical threshold value (E_c) of overall transmembrane potential (sum of the externally induced and resting potentials), breakdown of the cell membrane (i.e. electroporation) occurs with formation of micropores that increase permeability and may eventually result in membrane rupture [19], as schematized in Figure 1.



Figure 1. Mechanism of PEF action.

The final effect on a specific membrane wall, therefore, depends on the strength of the electric field and intensity of treatment, making the membrane rupture reversible or irreversible. The phenomenon is reversible if the increase in permeability is temporary, and the wall returns to the initial conditions upon termination of its exposure to the electric field, evolving along pore formation, expansion, and resealing phases. Such a process is mostly used in biotechnology applications (e.g., for DNA transfer into bacterial cells). Breakdown is irreversible if the cell dies due to prolonged, high exposure to the electric field. The critical intensity of electric field in both cases is influenced by process parameters, cells' physical-chemical parameters (conductivity, extracellular medium ionic composition, density, osmotic pressure, and others), and conformation (type, size, shape) [20]. Process operating parameters for electric field strength are: treatment time, pulse shape and width, number of pulses, specific energy input, and repetition frequency. All these must be adjusted according to the nature of the substrate under treatment and the desired effect. Generally, larger pulse widths increase tissue disintegration at constant pulse number, and larger number of pulses at shorter widths result in greater efficiency at constant total treatment time. Lower frequencies result in greater disintegration at constant pulses number [21]. A PEF system usually consists of a high voltage power source, a function

generator, a switch circuit, and a proper treatment chamber. A commercial, tube-type PEF apparatus is shown in Figure 2. Design of PEF systems was addressed by Kovacic et al. [22].



Figure 2. PEF tubular prototype apparatus, and scheme of working principle.

3. PEF Application Areas in Wastewater and Biomass Residuals Valorization

Wastewater treatment biological residuals (sewage sludge) consist of excess biomass produced during biological treatment processes. Sludge matter contains organic matter, nitrogen, phosphorus, micronutrients, and some residual contaminants (pathogenic organisms, toxic metals, recalcitrant organic compounds). Methods for safe sludge disposal incur considerable costs (they may contribute 30–50% of the total operation costs of wastewater treatment processes [23]) and may cause considerable environmental impacts [24]. On the other hand, waste sewage sludge still contains valuable resources, like nutrients and energy, that can be recovered through a variety of approaches.

In recent years, a new class of mixotrophic wastewater treatment processes based on algal cultures, where light and CO_2 are utilized in addition to organic carbon compound for biomass growth, has been increasingly studied. The interest in algae as production organisms in wastewater treatment is not only due to the fact that algae-based systems can simultaneously remove BOD, N, and P, but also to potential substantial advantages compared to traditional bacterial systems in biofuel production and CO_2 emissions mitigation [25], and as feedstock in biorefineries, or other applications [26].

Waste lignocellulosic biomass (LCBs) from plants and crop residues is a globally available, abundant, and potentially carbon-neutral sustainable feedstock containing sugarrich platforms. LCB is already widely used for biogas production and may be converted to other biofuels or originate recovery of specialty products by biorefinery. The nature of the lignin-polysaccharide matrix, however, makes it recalcitrant to biodegradation and compounds extraction [27]. The identification of proper pretreatments is therefore an essential step in LCB energy conversion and materials recovery.

This section summarizes significant reported experiences in the use of PEF technologies in resources recovery from wastewater-originated biomass residues, as depicted in Figure 3.



Figure 3. Possible PEF applications for biomass and bioresources recovery.

3.1. Biomass-to-Biogas Generation Improvement

Wastewater sludge and biomasses treatment practices emphasize the use of biosolidsembedded chemical energy for the recovery of usable energy forms. Of particular interest is the generation of methane-rich biogas through the fermentation of organic materials by bacteria in anaerobic digestion (AD) processes, one of the mainstream sludge processing technologies from the perspective of energy recovery. Wastewater sludge (including mixed algal-cell biological substrates), as well as other biomasses, such as waste-grown microalgae and lignocellulose are considered biofuel feedstock to all effects, with high potential for biogas generation. While AD is a standard application for biogas production from sludge in biological wastewater treatment facilities, there are more than 7000 algal wastewater treatment ponds systems in the US alone, and these could potentially yield as much as $0.25-0.50 \text{ L CH}_4/\text{g VS}$ when treated with an 11-day HRT mesophilic AD [28]. Combined activated sludge/algal culture facilities are also being designed and operated to treat domestic wastewater to take advantage of synergic effects of CO₂ uptake, O₂ production, and methane generation from AD of residuals [29]. In addition, with proper pretreatment focused on improving cellulose and hemicellulose accessibility, lignocellulosic biomasses (LCBs) chemical energy could also be converted to biogas.

Biogas, among the other renewable biofuels, is easily applicable to static and mobile uses [30] and is targeted by European policies as a key component of sustainable circular economy [31]. For AD to be efficient, ready availability of organics contained in the substrate mixture to the bacterial archea contributing to the process is critical, yet most organics are initially contained within substrate cells or complex macromolecules in the process feed and often not readily accessible to microorganisms. Many pretreatment methods have been investigated to enhance AD biogas production with minimal economic costs, energy consumption and without adverse environmental impact. Ideally, an optimal pretreatment process should require no or minimal heating (to avoid the need for large reactors).

Solubilization (hydrolyzation) of organic compounds in cells has been indicated as one of the most common process rate-limiting steps [32,33]. During hydrolyzation, high molecular weight organic compounds (proteins, carbohydrates, and triglycerides) are degraded into simpler molecules (amino acids, monosaccharides, disaccharides, fatty acids) that are thus made available for subsequent degradation. Different solubilization methods such as thermal, mechanical, chemical, irradiative, biological, or combinations thereof, are therefore often employed prior to an AD process. Biological hydrolyzation enhancement requires larger reaction volumes in the AD line [32]. Among recently proposed technologies, microwave (MW) irradiation can enhance hydrolyzation by sludge disintegration; however, major disadvantages of the MW destruction method are the high energy consumption and equipment costs, possibly resulting in a negative overall energy balance (methane produced minus energy appli6ed for pretreatment) of up to 300 kWh/ton sludge [34]. Subsequent studies showed that, in order for MW irradiation be effective, additional sludge pretreatment (e.g., chemical deflocculation with sodium citrate, or others) could be required prior to it [35].

PEF is considered a non-thermal process (due to Ohmic heating effects, temperature usually does not exceed 40 °C) [36], indicated as effective in accelerating the hydrolysis step, since the effect of the electric field on raw substrates leads to the increase of bioavailability of entrapped organic compounds, leading in turn to more efficient AD at shorter sludge retention times when applied as a pretreatment step [37].

The earliest studies concerning PEF application method in biogas production improvement are relatively recent (early 2000s). Earlier studies investigated the process' effect on the release of dissolved organic carbon (DOC) from biological sludge and COD uptake, and the impact of treatment intensity on the conversion of solids and refractory organics to more bioavailable soluble and colloidal forms (readily biodegradable COD, rbCOD). It was shown that methanogenic conversion increased consistently (80% for pig manure, 100% for waste sludge) at hydraulic retention time (HRT) between 25 to 30 days, with process application intensities up to 40 kWh/m³ [15]. PEF pretreatment at levels of 34 kWh/m³ increased methane by 33% and COD removal up to 18% while CH₄ production from co-digestion of landfill leachate and agro-food industry slurry increased by up to 44 and 8% with treatment at 50 and 15 kWh/m³, respectively. COD removal efficiency increased by up to 100% for landfill leachate, and 17% for fruit/vegetable slurry, compared to non-preprocessed substrate [38]. These results are comparable to those obtained by preprocessing sewage sludge with ultrasonication (US) or MW, which achieved biogas production improvement of 27% and 20%, respectively. An energy balance of the entire process, however, showed in both cases a negative net energy production of -15.3 kWh/t and -18.1 kWh/t, respectively, indicating that both processes were not energetically sustainable [39].

PEF has also been tested in algal AD's biogas production: *Chlorella vulgaris* was subjected to PEF intensity in the range 2–150 kWh/m³ with maximum solubilization of soluble COD improving to >830% and enhancement in biogas production of around 27% at process intensity of 5.4 kWh/m³, which increased by as much as 110% at 35 kWh/m³ [40].

PEF applications to LCB for biogas production enhancement are not many, mostly at laboratory scale; however, all studies showed an enhancement of CH_4 production and a positive energy balance. Lindmark et al. evaluated PEF technology pretreatment for improving biogas production from ley crop silage with batch AD. CH_4 yield increased by 16%, at 30% reduced digestion time in comparison to untreated substrate at electric filed application of 96 kV/cm [41]. Energy balance showed that the pretreatment produced twice the output (in the form of methane) compared to the electrical energy input.

PEF pretreatment of hybrid *Pennisetum* stems induced superior biogas production efficiency, higher cumulative biogas production (by 27%), and maximum CH₄ concentration in biogas [42]; pretreatment of harvest residues enhanced both biogas and CH₄ yield by 18 and 16%, respectively, in corn stalks (E = 0.935-1.664 kV/cm for 10 min), 18 and 17%, respectively, in soybean straw after pretreatment (E = 0.760-1.354 kV/cm, duration 30 min) in soybean straw. The calculation of the energy balance showed the highest energy gains from pretreated AD substrates of 13.30 kWh/t for corn stalks, and of 14.95 kWh/t for soybean straw [43].

Comparison among different pretreatment methods (freezing, alkaline or acid treatment, ultrasounds, and PEF) on AD biodegradability of the LCB components of grape pomace in terms of CH₄ production in large scale, continuous mode digesters showed that PEF resulted in maximum increase of cumulative CH₄ yield of 4%, and hydrolysis constant increase by 14%, although it did not give the best overall results [21]. Table 1 summarizes PEF applications for biogas production augmentation from various biomasses.

Feedstock	Description	
Sewage sludge, Pig manure	E = 24.5 kV/cm, HRT = 0.01 s, TI = 4.0–19.8 kWh/m ³ . Solubilized ~10% of total COD, soluble COD increased from ~20 to >1000 mg/L. Increased methane production after 25–30 days: 80% for pig manure, up to 100% for WAS.	[15]
Landfill leachate and vegetable slurry	E = 20 kV/cm; TI = 15, 30, 50 kWh/m ³ . CH ₄ production from landfill leachate increased up to 44% and COD removal by 100% at the highest TI, production from vegetable slurry increased by 7% at lowest TI, with COD removal of +17%.	[37]
Sludge (primary + secondary) from wastewater treatment	TI = $30.0-35.8 \text{ kWh/m}^3$. CH ₄ production increased by 33%, COD removal by 18%.	[38]
Biological sewage sludge	E = 5.88 to 14.7 kV/cm, Energy input = 150–280 kJ/L. Pretreatment efficiency increased with E level. Increase of T also increased pretreatment efficiency.	[44]
Biological sludge (60% WAS, 40% primary) from WWTP	E = 8 to 30 kV/cm. Biogas yield improved by up to 20%, and reduced sludge foaming during AD.	[45]
Thickened WAS	V = 20 kV (coaxial and multiple ring electrodes) Biogas yield increased by 150%.	[46]
Primary WWTP sludge	V = 30 kV; TI = 33 kWh/m ³ . Increased AD production of CH ₄ by 8%, of VFAs by 7%.	[47]

Table 1. Reported PEF application in biogas augmentation from various biomasses.

Later studies investigated PEF pretreatment impact on the anaerobic microbiodome's structure and function following the observation of methanogenic generation increase. Results showed significant shift in phylotypes after pretreatment (e.g., increase of *Deltaproteobacteria* and *Spirochaetes; Methanoculleus* decreased from 66% to 32%, and *Methanosaeta* increased from 22% to 55%, leading to more efficient acetate utilization) within the digester [48]. Subsequent research also revealed that PEF-pretreated anaerobic sludge consisted of more highly diversified bacterial community, the activity of which was stimulated by the presence of more bioavailable organic matter [49].

The optimization of the process focuses on economic aspects, i.e., the additional biogas yield compared to additional energy consumption, reduction of HRT, leading to smaller AD reactors, and final amounts of digested biosolids. Optimization, however, is a challenging task due to many variables and parameters involved, previously described in Section 2, which can significantly influence the final outcome in terms of improved methane yields. Process parameters must therefore be chosen with specific consideration to each individual condition (substrate characteristics, reactor type, and expected effects).

3.2. Enhanced Phosphorous Recovery

Phosphorus (P) is a finite, irreplaceable resource extracted from a non-renewable mineral (phosphate rock); the rise of global population and living standards expectations is anticipated to drastically increase the demand for phosphorus due to the unavoidable need to produce more food for humanity. Current studies suggest that the world may deplete the known available phosphorus reserves in less than 300 years from now [50].

About 16% of mined P ends up in the human diet, and eventually in their excreta, therefore wastewater and excess biological sludge, where P content may reach up to 7-10%of the total solids under an extended biological P removal process (EBPR) [51], show great potential for P recovery. Chemical precipitation recovery [52] is an established process for producing phosphorus-containing minerals, such as struvite and other bioavailable P compounds from wastewater [53] and wastewater residues [54], which would be suitable for reuse in the fertilizer industry. Release of cell-immobilized P into solution, leading to subsequent crystallization, is the limiting step in these processes. Cell-stored P can be released by anaerobic fermentation, hydrolysis, or various pretreatments that disrupt the cells' structure, either physically (e.g., heat, irradiation, sonication), chemically (e.g., acid/alkali attack), or by combinations of the above. Among physical processes, PEF can not only break the floc structure, but also the cell membrane to promote the release of cell-contained substances, including phospholipids [16]. The specific release mechanism of P forms under PEF treatment was investigated by Hu et al., pulsed discharge voltage of 40 kV, pulse frequency of 400 Hz, and forming capacitance of 4 nF, with electrodes separation distance of 5 mm were used [54]. Compared with raw sludge, PEF-pretreated sludge significantly enhanced the release of poly-P, indicating ongoing electroporation of cell membranes. Tests indicated that P release could be enhanced by 26.7% as soluble ortho-P by PEF followed by anaerobic fermentation, compared to fermentation alone, with PEF contributing 42.2% of the additional total soluble ortho-P increase. It was also shown that the release of poly-P from sludge could be enhanced by destruction effect of the sludge flocs occurring within the process. Total suspended solids removal rates of 23.8% and 34.3% of volatile suspended solids, respectively, were determined for the PEF-pretreated solid phase, suggesting that soluble organics production and sludge reduction were also enhanced by the process. The process' energy demand was determined as 0.12 kWh/Ltreated, achieving specific dissolution of 157.5 mg P. Compared to other pretreatment processes, PEF had superior P release efficiency than microwave irradiation (which induced P release of 50 mg P/L), which required similar investment costs and higher energy consumption than PEF, and acid/alkali pretreatment (with release effect of 60 mg/L at pH = 10, and 120 mg/L at pH = 3, respectively) [54]. Although conceptually simpler and less time consuming, chemical destruction involves high chemical costs and the increased risk of corrosion to process equipment.

PEF processing for release of sludge cells' stored phosphorus has some other important advantages compared to the different conventional methods applied: for example, heavy metals and other pollutants in the sludge do not pass into solution (acidification is not applied), hence pre-treatment for their removal prior to struvite precipitation is not required. Appropriate solution conditions for optimal removal are however necessary and could require sophisticated assays for proper identification [55]. PEF may thus be considered a promising pretreatment for P recovery and for the reduction of sludge quantities.

3.3. Sludge Dewatering and Volume Reduction

As an essential aspect of sustainable sludge management, waste sludge volumes (and mass) should be minimized prior to further treatment or biosolids reuse. Various technologies have been developed to this purpose, which are applicable either in the liquid treatment or in the sludge processing lines [56]. Sludge has a complex composition, particularly with reference to extracellular polymeric substances (EPS), mainly composed of large molecules' bacterial secretions, deriving from cell lysis and molecular hydrolysis, with primary components consisting of proteins and polysaccharides. Many functional groups of these compounds can form strong hydrogen bonds with water molecules [57]. Incineration is a widely used disposal method for wastewater treatment residuals, and other thermal processes (gasification, pyrolysis, hydrothermal carbonization) are also used [58]. These can recover energy from the sludge's embedded components as heat, gas (py-gas), solids (biochar), or liquids (py-oil) [59], and other recoverable residuals, that may feed local Circular Economy circuits [60]. A severe technological limitation of these processes lies in the fact that the residuals' water content must be well below 30% (ideally, closer to 15%) otherwise the energy required for water evaporation (theoretically 2270 kJ/kg) will significantly affect their energy balance. Hence, sludge dewatering methods that alter the composition and structure of EPS to promote easier release of bound water have been developed, including acid/alkali treatment, bioflocculants dosage, ultrasonic irradiation, heat, and pulse electric field treatment [61].

Effectiveness of the PEF process depends on achieving uniform packing of the treated medium (sludge or other) between electrodes, under proper conditions. An excessive presence of free high conductive liquid would increase electrical energy losses, but low values of external moisture could also limit the effect of the process due to lack of interparticle contact. Increase of PEF efficiency was observed after pre-compression of the substrate, with removal of some excess free liquid in the initial steps of the process: the applied pressure damages punctured cells, enhancing moisture migration and depressing cell resealing processes [62].

Low, direct current electric field applied to sludge also induces an electroosmotic flow phenomenon in the matrix; in turn, this promotes extra water removal from the sludge, increasing the final "cake" solids content. In addition, electrical field application induces electrokinetic phenomena in the matrix, such as electromigration and electrochemical reactions at the electrodes, and these also positively affect, directly or indirectly, dewatering as result of the release of intra- and extra-cellular constituents and bound water. PEF can thus be used as a preprocessing step in sludge dewatering, since disposal costs of dewatered sludge decrease proportionally to the decrease of water content. Reducing energy consumption for sludge dewatering (about 2500 kWh/ton d.w.) is a key necessity for sludge disposal, in view of limitations foreseen for some current options, such as landfilling and land application [63], and the feasibility and efficiency of common alternative processes of sludge [64]. Return flow from PEF-dewatered sludge has significantly higher soluble rbCOD content, up to 160% more [15] than under normal process operation (e.g., centrifugation alone), and thus a higher recoverable energy content in AD processes. Since sludge disintegration technologies are also effective in pathogen inactivation, except for spores and viruses [65], dewatered sludge can be used for land applications where appropriate.

3.4. Sustainable Biorefineries

The global market share of biofuels, including biodiesel, bioethanol, biogas, and biobuthanol, is expected to grow significantly in the next years, due to their lower negative environmental impact compared to fossil fuels, and to strong regulatory drives by most industrialized countries. In particular, the so-called 3rd biofuels generation, i.e., those derived from microalgae, is drawing particular attention since it became apparent that algae could be capable of much higher yields than other traditional feedstocks, producing oils (lipids) that can be easily refined into diesel, gasoline, and even jet fuel components by means of an array of technologies such as pyrolysis, dilution, micro-emulsification, transesterification, and others [15]. Chemical reaction of triglycerides and alcohol supported by catalysts such as sodium hydroxide is among the processes that can be used, which are referred to under the general term of biorefinery. In addition to biofuels production, microalgae showed to be a possible alternative source of proteins to meet future global protein requirements [66]. Microalgae, in fact, show a high content of valuable compounds such as lipids, proteins, polysaccharides, antioxidants and pigments (>50 g/100 g d.w.), have high biomass growth rate and productivity (three to five times higher than crops like corn, canola or soy), and they offer the possibility to be grown on non-fertile land (without competition for land with food crops), in controlled conditions, even using wastewater as substrate, in any season [67].

It has been postulated that an attentive biorefinery exploitation of a broader spectrum of microalgal constituents would enable a rapidly increasing growth of Green Economy, however, it has been pointed out that production costs should be reduced at least by an order of magnitude for algal products to be economically competitive [68]. Algal cultivation delivers algal solutions at 0.05–0.075% to 0.3–0.4% d.m. for open pond and closed systems, respectively. One of the greater limitations to the economic sustainability of these processes is related to algae drying and compounds extraction, which consume large amounts of energy and account for approximately 30% of production costs [69]. Dewatering and drying technologies are usually adapted from similar wastewater sludge processes and include mechanical/physical methods (i.e., centrifugation, pressure, vacuum or membrane filtration, sonication, high pressure homogenization, microwave irradiation, supercritical fluid extraction, flocculation, solar or drum drying), and non-mechanical ones, such as chemical (solvent extraction, osmotic shock) and/or enzymatic. An economic evaluation showed that operational and energy consumption costs for these are in the range 0.2–5 €/kg algae [68]. Algal cell walls are thick and rigid due to the existence of covalent bonds, hydrogen bonds and van der Waals forces interaction among the molecules. This makes algal cell disintegration and intracellular compounds extraction difficult and costly and identifies these steps as the main bottlenecks in the industrialization of microalgae exploitation in biorefinery [70]. Table 2 summarizes results achieved in lipid extraction from various algal species by conventional and other technologies, including PEF.

Method	Operating Conditions	Microalgal Strain	Lipid Extraction Yield (% wt)	Reference
Ultrasound + solvent	Ultrasonication in ice/ water bath for 20 min	Chlorella minutissima, Thalassiosira fluviatiis, Thalassiosira pseudonana	15.5–40.3	[71]
Ultrasound + solvent	US: 40 kHz, 2.68 W/m2, 25 °C 2-step extraction with: 1)CH ₃ OH + CHCl ₃ /US _{40min} or CH3OH/US _{3min} or C ₃ H ₇ OH/US _{4min} 2)CHCl ₃ + Na ₂ SO ₄ /US _{20min} or CH ₂ Cl ₂ /US _{27min} or C ₆ H ₁₄ /US _{56min}	Chlorella vulgaris	2.2–52.5	[72]
Ultrasound + Soxlet	40 kHz, 2.68 W/m ² , Soxhlet for 8 h with acetone	Chlorella vulgaris	1.8	[72]
MW + solvent	2.45 GHz, 400 W _{30min} CH ₃ OH + CHCl ₃	Chlorella pyrenoidosa	19.03	[73]

Table 2. Summary of conventional and novel methods for lipid extraction from algal cells.

Method	Operating Conditions	Microalgal Strain	Lipid Extraction Yield (% wt)	Reference
MW + solvent	$\begin{array}{c} 500 \ W_{5min}, 65 \ ^{\circ}\text{C} \\ \text{Various extraction methods (2 stage) with} \\ \text{CH}_{3}\text{OH}, \text{CHCl}_{3} + \text{Na}_{2}\text{SO}_{4}, \text{CH}_{2}\text{Cl}_{2}, \\ \text{C}_{3}\text{H}_{7}\text{OH}, \text{C}_{6}\text{H}_{14} \end{array}$	Nannochloropsis sp. Tetraselmis sp.	4.2–8.4 5.4–8.2	[74]
Osmotic shock + solvent	10% NaCl 48 h, then CHCl ₃ + CH ₃ OH	Botryococcus sp. Scenedesmus sp./Chlorella vulgaris	~11 ~8	[75]
Solvent extraction	CHCl ₃ + CH ₃ OH	Isochrysis galbana, Nannochloropis gaditana, Nannochloropsis sp., Phaeodactylum tricornutum	17.8–30.2	[76]
Bead-beating + Supercritical CO ₂ extraction	1500 rpm for 5 min. SC-CO2: 306 bar, 60 °C, 6 h	Pavlova sp.	17.9	[77]
Enzymatic	37 °C for 2 h with: Viscozyme or Papain or Proteinase K or Driselase	Phaeodactylum tricornutum Thalassiosira pseudonana	92–104 88	[78]
Enzymatic + solvent	Papain, 37 °C, 2 h plus C ₇ H ₁₆ or C ₇ H ₁₆ + C ₃ H ₇ OH	Phaeodactylum tricornutum	56–96	[78]
Enzymatic + solvent	Cellulase 60 °C, 4.6 pH, 72 h then n-C ₆ H ₁₄ , 28 °C	Chlorella sp.	10.6	[79]
Electric Field lysis	0.3A, 60min, 14.3–30.7 V/cm	Chlorella vulgaris	2.08–3.7	[80]
Fenton Reaction + solvent	$H_2O_2 + n - C_6H_{14}$	Chlorella vulgaris	9.24–17.37	[81]
PEF	2.7 kV/cm, W = 14.4 kJ/L	Chlorella vulgaris	22	[82]
PEF	35 kV/cm, 200 kJ/kg _{ss}	Auxenochlorella protothecoides	22	[10]
PEF + solvent	>35 kWh/m ³ , Isopropanol	Synechocystis PCC 6803	25–75	[83]

Table 2. Cont.

Algal extraction technologies reported and tested include freeze-thaw, bead-milling, ultrasonic, chemical, and osmotic shock. Application of these methods are however limited due to high energetic demand or operational difficulties. Wet extraction methods are normally cheaper than dry ones (require no energy to dry biomass), however, research showed that chemical wet lipid extraction with solvents (e.g., chloroform: methanol) can show efficiency reduction by up to a third [84]. Unlike in traditional cell disruption technology, which completely disintegrated cell wall membranes and released all cell components, electroporation can enable highly selective extraction of intracellular products, maintaining their original quality and subsequent fractionation of pure extracts [85].

In addition to energy uses, many microalgae-derived products are also sold to commodity markets, competing with products from conventional sources. The potential market has been conservatively estimated to be in the range of \$500–1000 million/year with rapid growth and consists of two main areas: biofuels and "everything else", which includes food (for both animals and humans), nutritional supplements (e.g., omega-3), cosmetics, fertilizers, and many other specialty chemicals, with the non-biofuels category actually commanding the highest commercial prices. Decreasing extraction costs has been recognized by the algal industry as one of the most significant challenges to these products' commercialization [86]. In the last years, sales of algal-based products have grown significantly, and at the same time wide industrial interest in PEF application has arisen, with special focus on increasing the cost-effectiveness of algal-derived products extraction. Similar to the key requirements for sludge processing technology, lipid and other constituents' extraction technologies must include high efficiency (in terms of time and energy), non-reactivity with the compounds of interest, environmental safety, and relatively low cost (in terms of equipment and operation). Extraction of specialty products from lignocellulosic biomasses from plants and crop residues (e.g., phenolic compounds, and polyhydroxyalkanoates, PHAs, one of the most promising degradable alternatives to plastic from fossil sources) by biorefinery is becoming industrially attractive, as these are widely available and potentially carbon–neutral sustainable feedstocks. LCB is already widely used for biogas production in co-digestion facilities, and may be converted to other biofuels or market-valuable extracts. LCB is composed of carbohydrate polymers (cellulose, hemicellulose) linked to an aromatic polymer (lignin); its complex structure makes its depolymerization, necessary to perform valuable compounds recovery, quite challenging. PEF-assisted polyphenols extraction from spruce bark biomass at electric field exposure of 20 kV/cm at pH 12 increased yields eightfolds compared to non-PEF-assisted samples, PEF being a good alternative to high temperature solvent extraction and grinding processes, with lower reported energy consumption (3.2 kJ/g vs. 8.75 kJ/g) [87]. Table 3 summarizes reported PEF applications in biorefinery of various feedstocks.

Table 3. Reported PEF applications in biomasses biorefinery.

Feedstock	Purpose	Description	
Algae	Protein extraction	E = 3-34 kV/cm	
A. prototnecoues, C. ouiguris, N. salina		Protein extraction yields: 3.5–5 μg protein/100 μL	[88]
FoodcropsHigh value productsresidualsextraction		E = 5–20 kV/cm >50% energy saving compared with traditional methods, extraction yield of polyphenols increased by 150%	[89,90]
LCB	Biofuel production	E = 8-10 kV/cm	[91]

On a final note, PEF application for the improvement of algal biomass cultivation was reported: genetic modification (delivery) assisted by pulse electric field was used to obtain stable transformants of both wall-less and walled strains of microalgae *Chlamy-domonas reinhardtii*, *Chlorella ellipsoidea*, and *Dunaliella salina* [92]. This technology was also implemented to optimize industrial microalgae production by controlling protozoa contamination (predators that can substantially jeopardize algal productivity) of an industrial microalgae photobioreactor. A contaminated culture was treated at 900 V/cm with 65 μ s pulses of 50 Hz, reducing the active protozoan population by 87% after 6 h, and completely after a few days of normal cultivation, showing that PEF is effective on the selective elimination of protozoa by inflicting on these organisms cell rupture, growth inhibition, or death without affecting microalgae productivity [93].

4. Discussion

PEF technology appears to have substantial promises in improving organic residues (wastewater and biomasses processing) management, even if data available are currently limited to the most interesting commercial sector of microalgae biorefinery. Data are not yet conclusive, with occasionally contrasting results: in one application (E = 9.6 kV/cm) for *C. zofingiensis* lysis, a cost of \$2.69/barrel was estimated for pretreatment, over an order of magnitude lower than the cost of a thermal process (~\$40 USD/barrel) under the same assumptions [94]. Assuming that subsequent compounds' extraction costs are similar after either process, PEF would result over an order of magnitude cheaper. Other researchers, however, estimated that PEF efficiencies in terms of absolute yield and energy input were currently lower than those of another established process, such as bead milling [95]. Process efficiency seems to be affected by the specific experimental approach (substrate, operating conditions, and objective), with a wide range of results obtained, as summarized in the tables above.

Beside some apparent advantages, PEF technology also has a number of potential limitations, the most important one being the high initial capital investment. Although economic issues are seldom addressed in published literature, a few sources indicate that commercial PEF units manufactured for industrial scale (mainly food industry, medical, or biorefinery) applications are available at prices ranging from a few tens to hundreds of thousand dollars [22]. Furthermore, many technologies that look promising when tested at the small scale in new fields may not be effective or sustainable at large scale in continuous processes [96]. It should also be remarked that most studies focusing on biogas production or extraction processes improvement do not include the determination of realistic specific energy balances (e.g., kWh/m³ or t sludge). Usually, these estimates refer just to the pretreatment itself and do not account for the process as a whole, which may be affected by other inputs [97].

5. Conclusions

This paper summarized studies on the application of pulsed electric field technology for wastewater and biomass residues' improved valorization for renewable fuels production and recovery of nutrients and value-added products. PEF pretreatment leads to release of intracellular embedded substances from substrate cells, facilitating the contact between these and external bacteria for conversion to methane or their extraction for further processing. These PEF applications represent an attractive approach for future valorization of sewage sludge and biomasses, since they have demonstrated great promises in the improvement of existing processes' yields, however, they also constitute a great challenge as PEF pretreatments, now commonly used in the food and medical industry and in some biorefinery applications, are still in infancy in the specific residues valorization sector. Commercial equipment has been successfully developed for food industry applications (mils and juices pasteurization, potato chips, and other snacks pre-processing), which may have economic margins similar to those of the residues recovery sector, however, it may not be suitable for the latter due to the properties of the media involved. Further research and development on specific equipment adapted to each substrate and on its field performance is needed.

The determination of closed energy balances for the entire processes involved, and not limited to the pretreatment phase itself, is also needed on each specific case study in order to assess the impact of PEF processing on residues' valorization.

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Conflicts of Interest: The author declares no conflict of interest.

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