

Electrocoagulation Process for Microalgal Biotechnology - A Review

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Abstract: As an electrotechnology, electrocoagulation (EC) is founded on the uninterrupted exercise of an external electric field across specified semi-conductive electrodes. This technique may be viewed as a side of a large domain of biotechnological methods and perceived cost-effective and environmentally-friendly in terms of the less intensive application of non-renewable resources and elevated degrees of energetic performance. From this point of view, EC is an encouraging treating system to control several of microalgae's utilization restrictions. Using electric field-founded technologies may include upstream (i.e. electroporation for genetic transformation, inactivation of culture contaminants, and improvement of growth kinetics) and downstream processes (e.g. harvesting and extraction methods). This review gives a thorough information of the present situation of the explicit usage of such methods on microalgal biotechnology, also following tendencies and defies concerning expansions in EC to be used to microalgae manufacturing utilization. Like other electrotechnologies, EC remains a viable process usable in microalgal biotechnology even if it is based on the destruction of the cells. However, more researches should be planned in the perspective of a large industrial usage of this electrochemical technique.

Keywords: Electrocoagulation (EC), Microalgal Biotechnology, Electric Field, Electrodes, Electrochemistry, Algae

1. Introduction

Microalgae are a very varied category of microorganisms, symbolizing one of the more ancient manifestations of life on Earth [1-4]. Such microorganisms are described as primitive plants (thallophytes) – not presenting roots, stems, and leaves – including unicellular plants (Chlorophyta), bacteria (Cyanobacteria), diatoms (Chromalveolata) and protists (Chromista) that can be encountered firstly in marine and freshwater mediums [5-7]. On the other hand, with higher plants, microalgae do not require a vascular system for nutrient transport (absorbing nutrients directly), which grants a major benefit in the matter of energy performance [8, 9]. Considering the nutritional needs of microalgae, they could be viewed as autotrophs or heterotrophs following the source of carbon consumed for growth is inorganic mineral ions or organic compounds [10], respectively (Figure 1) [8]. The major versatility showed by microalgae let them grow on a large domain of mediums throughout the earth comprising under excessive situations of temperature, pH, light intensity,

and salinity [11, 12].

Regardless of the boundless profit-oriented possibility of microalgae (see Figure 1), these microorganisms persist considerably undiscovered as many millions of species are guessed to live [13-15]. The beginning of microalgae usage by humans happened around 2000 years ago when the Chinese initiated consuming them as a food source [12].

De facto, thanks to their elevated nutritional amount, particularly in terms of proteins, lipids, and carbohydrates [16], microalgae are still largely employed as a source of food in Asia [17-19]. Chlorella, Spirulina, Haematococcus, and Dunaliella constitute the larger part of the market, which may be sell in tablet, capsule, liquid, and powder forms or added to pasta, snacks, and drinks as nutritional complements or natural dyes [7, 17, 20-22]. When consumed, the high-protein tenor microalga Chlorella was observed to improve the development of intestinal Lactobacillus, at the same time Spirulina sp. and Dunaliella sp. may play the role of a strong anticancer agent thanks to their carotenoids content [23, 24]. Regardless of the considerable labors for leveraging microalgae as human food, linking them to “healthy” food,

the elevated formation costs and the rigorous food safety regulations have been seen to be restrictive to this mindset change [12]. Therefore, microalgae cultures have been first employed as feed compliments in the aquaculture food chain (e.g. larvae and juvenile mollusks, penaeid prawn, and crustaceans, as well as fish) enhancing immune system response, fertility and weight control in addition to promoting a healthier skin (e.g. more colorful) and lustrous coat [14, 25, 26]. Now, besides food and feed usages, microalgae are employed in chemical, biofuels, pharmaceutical, and cosmetics industries, either in whole-cell form or through their functional compounds [25, 27-29].

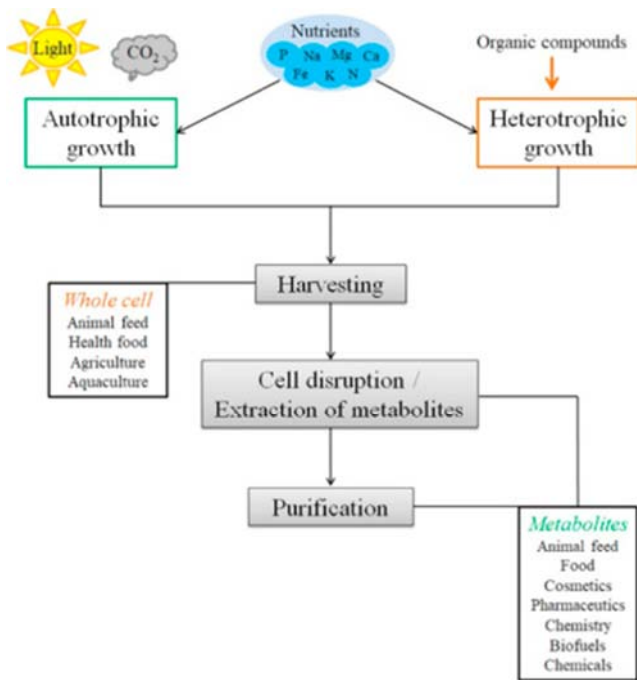


Figure 1. Microalgae formation stages and conceivable utilizations [12].

To decrease microalgae formation costs and thus prompt its popularized commercialization, planning could be applied emerging algal development in open or closed devices with CO₂ absorption (i.e. from consumed gases resulting from fossil fuel combustion) and/or wastewater treatment techniques, concealing nutritional requirements of microorganisms [8, 27, 29, 30]. This will not only participate to control problems implying greenhouse gases emissions (that is CO₂) and pollutants elimination from wastewater but as well form economic value employing wastes or by-products from other industrial methods [11]. Consequently, aside from enclosing ecological sustainability, like generation program is as well appropriate to employ microalgae cells in the role of raw material to generate not only a large domain of biofuels (like biodiesel and bioethanol), but as well elevated added-value bioactive chemicals (such as proteins, pigments, vitamins, antioxidants) [8, 31, 32]. Considering the benefit of the capacity of many microalgae for absorbing atmospheric nitrogen, viz cyanobacteria species, such microorganisms are usually employed in agriculture in the guise of biofertilizers [33]. Throughout absorbing nitrogen,

microalgae ameliorate soil fertility and its physicochemical features conducting to more elevated plant development yields. In addition, employing microalgae in the role of biofertilizer involves additional advantage for plants, which concerns the formation of various growth-promoting substances such as vitamin B12 [7]. A diverse technique for employing microalgae in the guise of biofertilizers concerns subjecting them to a pyrolysis operation conducting to the generation of solid charcoal residue (i.e. biochar) [12, 25].

The main obstacles that require to be controlled so as to augment the number of microalgae strains cultivated at the big level are elevated production costs – mostly because of the quantity of energy necessitated throughout development and downstream steps – and increased initial investment [11, 27, 34]. Therefore, several scientists propose that microalgae formation could turn into economically practicable at the manufacturing level when multiple product exploitation strategies are implemented across the biorefinery design [11, 25, 35]. Following this planning, the joint market value of these products may exceed the costs implicated in the production path and thus augment microalgae biomass investment [36]. Nevertheless, traditional extraction techniques such as homogenization, heating, or osmotic shock are usually realized and completely experimented with the objective of increasing the collection of one specific product only, even if it induces destruction or damage and following wastage of all the other high-added value metabolites that may be existing [12]. As a result, mild, inexpensive, low energy consumption and effective techniques, like pulsed electric fields (PEF), low or moderate electric fields (MEF), ultrasounds, and eventually the use of green solvents, such as ionic liquids and eutectic solvents, must be conceived, optimized and synergistically emerged, not only to keep the features of all the extracted matters but as well to encourage a sustainable and more environment-friendly method [35, 37]. This review will describe and discuss the potential usages of electric fields (EFs) applying at many steps of the microalgae formation procedure.

2. Electric Fields (EFs) Usage

The expanding attention on the benefits of EFs applying in biological devices as a company with the requirement for more performant and cost-effective techniques are motivating the attraction in investigation and utilization of electrotechnologies [38]. Such techniques incorporate the usage of an external EF with a fixed technological target [39-41]. Even with the technical capacity of such electric-founded methods has been for a long time admitted, functioning issues and the absence of basic comprehension concerning the techniques made late their usage at a trade level [42-44]. And so on, much endeavor has been placed into the expansion and optimization of such techniques conducting to full-blown and robust methods with elevated performances, which is especially real in the situation of extraction usages [45]. Throughout the 1970's and 1980's the technical enhancements turned electrotechnologies into

strictly realizable [46]. These days, electrotechnologies have been ramified into fresh processes and utilization, obtaining likely or efficient usage in all steps of microalgae formation (see Table 1 in [12]).

Applying an EF may conduct to various consequences and effective implementations following its proper electrical features (i.e. EF intensity, kind of electrical waveform and electrical frequency, contact period, etc.) [47]. One of the EF impacts that deserve more interest is electroporation. Presenting a cell to an EF generates a transmembrane potential in the cellular film. The performance of the EF is the consequence of the field strength exercised and the cell radius, implying the smaller the cell radius, the higher the EF to be imposed with a view to attaining the identical impact [48]. If the transmembrane potential surpasses some minimum amount (i.e. 0.2–1.0 V), the EF induces electro-permeabilization of the envelope. This impact may be brief or enduring and can conduct to an augmented flux of compounds across the envelope, cell deterioration and demolition [49]. A global view of the electroporation process is shown in Figure 2 [12].

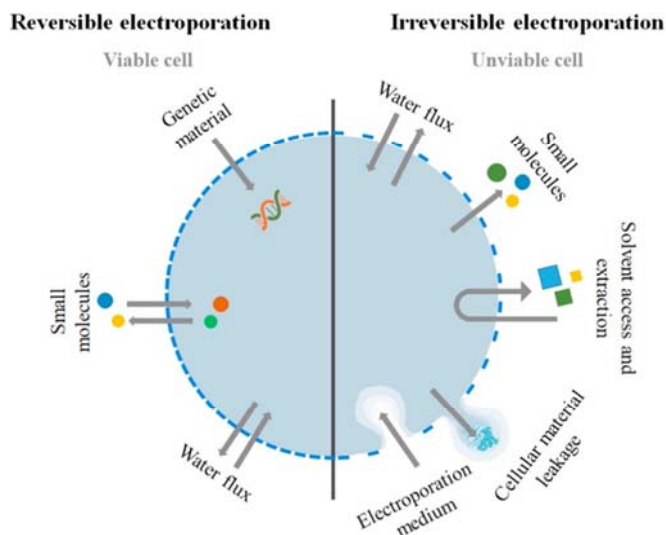


Figure 2. Diagram showing various electroporation degrees and their results [12].

A different result of applying an EF is heat production in the medium by the Joule effect. In this technique, frequently known as ohmic heating (OH), heat is formed immediately in the material itself, because the electric current overrides across the semi-conductive material. Heating is explained by friction happening throughout molecular agitation formed by the motion of charged molecules inside the material. The fast, homogenous and energetically performant (> 90%) heating given by the Joule effect has attracted attention in aseptic processing of liquid-particulate mixtures, processing residues, and extraction processes [46]. Like almost every particle found in an aquatic medium, cells and especially microalgae, possess an inseparable electrical charge. Such charges conduct to the existence of electro-kinetic processes if an EF is imposed [12]. Electrophoretic motions, in the existence of a continuous EF, or dielectrophoresis movement of dielectric particles under a non-uniform EF, suggest the

likelihood to electrically command the trapping, focusing, translation or fractionation of particles [50–52].

Notwithstanding such impacts being related to the existence of an EF, EF methods have to be linked to a specific group of variables and particularities with a view to be efficient and attain de facto significance [12]. Therefore, defining such techniques follows the variables group for the EF used and practical features implicated, more than on the EF itself. Many of the standards employed to specify various methods are the kind of the electric flow (i.e. alternating or direct, and pulsed or nonpulsed), the EF strength (V/cm), the intensity of heat loss, etc. [53]. Such indicators affect the impacts caused, the ranking of the methods, and are fundamental to describe the conception and working necessities of the system [54]. In this section, the substantial electrotechnologies used in microalgae generation and conversion will be consulted and described following their working variables and efficient or likely utilization. A general review of electrotechnologies global devices and standards is shown in Figure 3.

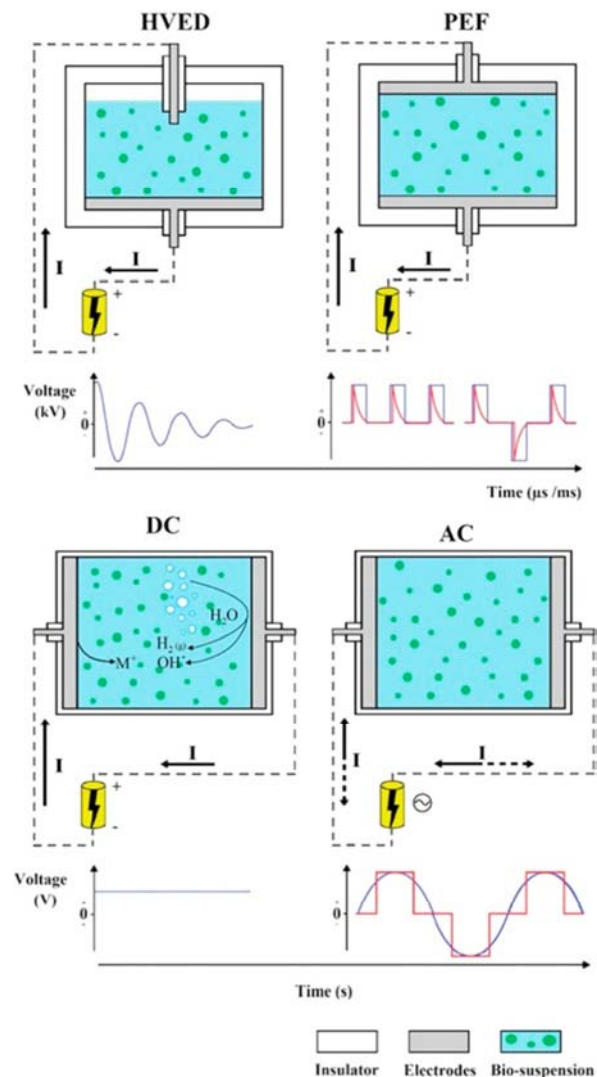


Figure 3. Diagrams of global systems and features implying electrotechnologies for High Voltage Electric Discharges (HVED), Pulsed Electric Fields (PEF), Direct Current (DC) and Altering Current (AC) utilizations [12].

Geada et al. [12] given a detailed explanation about the Pulsed Electric Fields (PEF), High Voltage Electric Discharge (HVED), Alternating Current (AC) usages. Since this review focuses on Electrocoagulation (EC), more details are presented for Direct Current (DC) in the following section.

3. Direct Current (DC)

Direct current (DC) may be described as the unidirectional flow of electric charges from one side to the other of two electrodes: the negatively charged (cathode) and the positively charged (anode). An immediate residence of such unidirectional flow of charges is the electrophoretic motion of charged particles to the opposite polarity electrode. Under the polarization of the electrodes, electrochemical reactions can happen, especially electro-oxidation of the electrodes and electrolysis of water, conducting to electrode corrosion (EC may be defined as accelerated electrode (particularly anode) corrosion) and gas bubbles emanation [55, 56]. Theoretically, the electrophoretic motion caused by a DC gives the potential of concentrating and separating microorganisms from the culture medium [57, 58]. EF intensity is a fundamental parameter in the technology as it influences the motion velocity of the cells [59]. Moreover, if the EF intensity is enough elevated, it can provoke permeabilization [60]. Nevertheless, the EF intensity to be exercised is restricted by

diverse indicators diminishing the above-mentioned impacts [61]. Elevated energy needs, excessive heat deposition and low durability of the electrodes are the major parameters lowering DC implementations to electro-kinetic separation [47]. The presence of electrochemical reactions may be utilized in the technique of microalgae harvesting because bacteria coagulation may be engendered by ions liberated from the electrodes (generally aluminum or iron) as they are submitted to oxidation [12, 62]. When the phenomena of EC happen, the flocs produced are displaced to the surface because the bubbles emanated via the water hydrolysis generate flotation [57, 63-65].

4. Using Electric Technologies in Microalgae Biotechnology

In microalgae handling, EF-based techniques (Figure 4) may be used from upstream (i.e. electroporation for genetic modification, deactivation of culture pollutants, enhancement of development kinetics) to downstream methods (e.g. harvesting and extraction processes) [53]. Geada et al. [12] assessed the status of the direct application of these techniques on microalgae biotechnology. Table 1 presents a brief description of employing electric technologies in microalgae technology.

Table 1. Usage of electric technologies in microalgae biotechnology [12].

Application	Description
Upstream applications	<p>Electroporation for genetic transformation</p> <p>The augmented attention in microalgae as a natural source of bioactive compounds and as micro-bioreactors has attracted novel planning for the intensification of their development and metabolites formation. If compared with other microbiological formation devices, microalgae possess numerous benefits. Usually, their biomass doubles within 24 h and may be developed either phototrophically or heterotrophically, rendering them applicable for the generation of therapeutic and industrially significant products [66]. The genetic manipulation of microalgal species has shown possibility to enhance many aspects of their cultivation and benefit compounds formation, like manipulation of the central carbon metabolism, antenna complex reduction or recombinant protein expression [67, 68]. A specific interest has been addressed to the generation of recombinant proteins of some microalgae species because they can be expressed from nuclear, mitochondrial and chloroplast genomes [66]. Thanks to such particularities, microalgae are viewed as interesting tools for the development of recombinant proteins and generation of bioactive compounds [69, 70].</p> <p>Control of culture's contaminants</p> <p>Biological pollution of microalgae cultures with predators or competing organisms may greatly affect productivity. It may conduct to losing completely the culture. Therefore, it is crucial to present efficient means for removing selectively such pollutants [71].</p> <p>Employing PEF as a cell permeabilization and disruption method may be helpful in selective cell removal (i.e. predator control) [72]. The field strength may be a selective mean to treat cells with diverse sizes, changing the performance with cell radius. Smaller cells require more elevated field strength to form identical impacts to those observed on bigger ones [73].</p> <p>Improved mass transfer – enhancing growth kinetics</p> <p>Stimulating biologically microbial cultures with a view to increase development and metabolic mechanisms has attracted interest and conducted to crucial findings. Moreover, employing EF has been shown efficient in enhancing microorganisms' development and productivity [74]. Sublethal range EF presents impacts on metabolic activity and cell membrane permeability of various organisms [75]. Among these influences are the lag phase decrease in <i>S. cerevisiae</i> by MEF and low intensity PEF [76, 77]. The augmentation of fermentation kinetics, proposing an influence of EF in the cellular machinery and an amelioration of mass transfer across the cellular membrane are reported [77].</p>
Downstream processing – harvesting	<p>Harvesting microalgae remains a defy to the commercialization of microalgal compounds because of their small size and weak colloidal stability in culture [78]. Many harvesting techniques like centrifugation, sedimentation, filtration, flotation and flocculation, or an integration of several of these, were largely investigated for microalgae [57, 63, 79]. Choosing the most convenient harvesting method is not only a function of the microalgae species but also of the kind and the level of the desired products [80]. Harvesting of small size microalgae or with small density usually necessitates longer processing times, especially if employing gravitational methods [63].</p>
Downstream processing – electroporation and extraction (High energy pulses and	<p>Certainly the most accepted and examined impact of EF handling is permeabilization of cellular material, rendering EF applications an encouraging choice to traditional cell permeabilization techniques (see Table 1 in [12]). Presenting bacteria to an external EF may modify the configuration of their envelope as it provokes a transmembrane charge exchange. As a result, the envelope loses its barrier function and becomes permeable, a phenomenon usually known as electroporation or electroporeabilization [72]. Such influences are linked to all EF usages because the threshold of transmembrane potential ($\sim 0.2-1$ V) is surpassed. Nevertheless, the</p>

Application	Description
moderate electric fields)	limitations in the exercised field strength in the situation of non-pulsed EF technologies restrict their electroporation impacts. Consequently, PEF is viewed as the main EF-related technology to help or encourage extraction of intracellular compounds [81]. The potential of using EF with many dozens of kV permits efficient cellular permeabilization and rupture even in small unicellular bacteria. Nonetheless, fresh means employing additional electrotechniques are presenting attractive trends in permeation of microalgae cells and extraction of their intracellular metabolites.

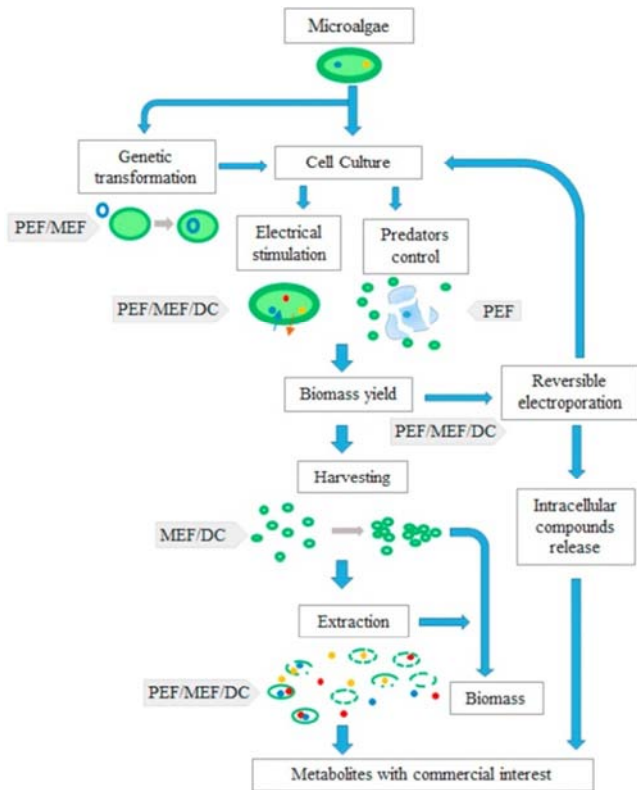


Figure 4. Global method diagram of microalgal biotechnology and the usage of electrotechnologies in diverse steps of the process [12].

5. Electrocoagulation (EC) for Microalgal Biotechnology

In classical coagulation/flocculation methods, pH variations and the injection of polymers or polyvalent metal ions like iron (Fe^{3+}) or aluminum (Al^{3+}) provoke charge neutralization and/or network generation [12, 82-84]. The fresh mean of electrochemical harvesting (ECH) technologies, founded on the concept of EC, electroflocculation and electroflotation, shows an innovative, efficient, and cheap process for harvesting of microalgae, decreasing (or avoiding) importantly the injection of chemical products [78, 85].

In EC, metallic ions are formed because of oxidizing metal electrodes, inducing the destabilization of colloids and the coagulation of biomass [79, 86]. Throughout EC operation, the oxidation of the anode happens under DC or low frequency AC field, which provokes electrode (especially anode) consumption and, therefore, necessitates periodic electrode substitution [63, 87]. Since the anode is used, an inexpensive material is recommended [88]. This consumption leads to metallic pollution of the harvested

microalgae, representing the primary inconvenient of the EC technology in matter of cost-efficiency [12]. Dewatering techniques that do not form trace metals are preferred because the products obtained do not suffer any market value depreciation [57].

Such electrolytic technologies of coagulation/flocculation have shown huge capacity and elevated performances [12]. Efficacious microalgae recuperations between 85% and 95% were attained within 1 h employing electrolytic flocculation with a power supply of approximately 5 V [79]. Identically, a 97.4% removal efficiency of *D. salina* after 3 min using electrolytic flocculation was also reached [12].

Like EC, electroflotation is an electrolytic process where gas (mainly oxygen from the anode and hydrogen from the cathode) bubbles emanating from the water electrolysis fix to the particulates in suspension and displace them up to the surface of the vessel [79, 89]. Generally, in this method the cathode is made from an inert material, which is electrochemically non-degradable, therefore decreasing the electrode substitution costs and pollution problems [12].

Integrating electrocoagulation–flotation (ECF) appears as an encouraging option to traditional microalgae harvesting thanks to the simplicity and potential of technique scale up. For the ECF process, coagulating ions are produced from the anode that are able to destabilize microalgae suspension (Figure 5). Moreover, the destabilized microalgae cells aggregate and constitute flocs (Figure 6), which are fixed by the microbubbles (H_2 and O_2) emanated from the electrodes [90]. Employing ECF for removal of microalgae from drinking or wastewater was studied in a few studies [90-94]; however, its utilization as a technique of collecting microalgae biomass needs more researches.

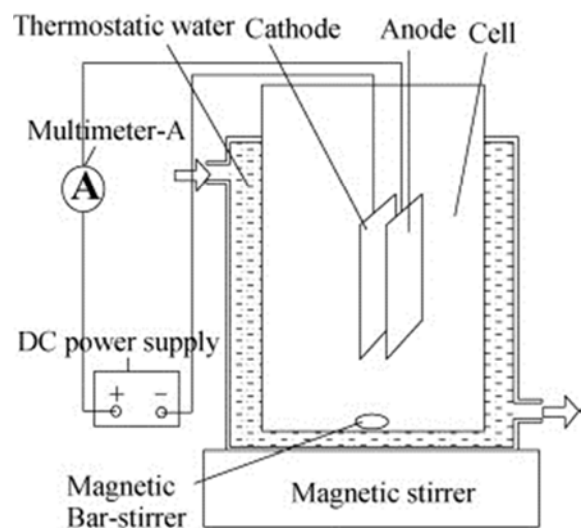


Figure 5. Schematic diagram of the batch ECF reactor system [90].

Kim et al. [95] focused on the integration of EC and electroflotation as a continuous cultivation and electrolytic microalgae harvesting technique. Following this innovative concept, one electrode is made of a sacrificial material and the other is made of an inert material. Via inverting the DC field and modifying the polarity of the electrodes, three operating configurations were experimented – continuous electrolytic microalgae harvesting with polarity exchange, electroflotation, and ECF – being the first the most efficient one.

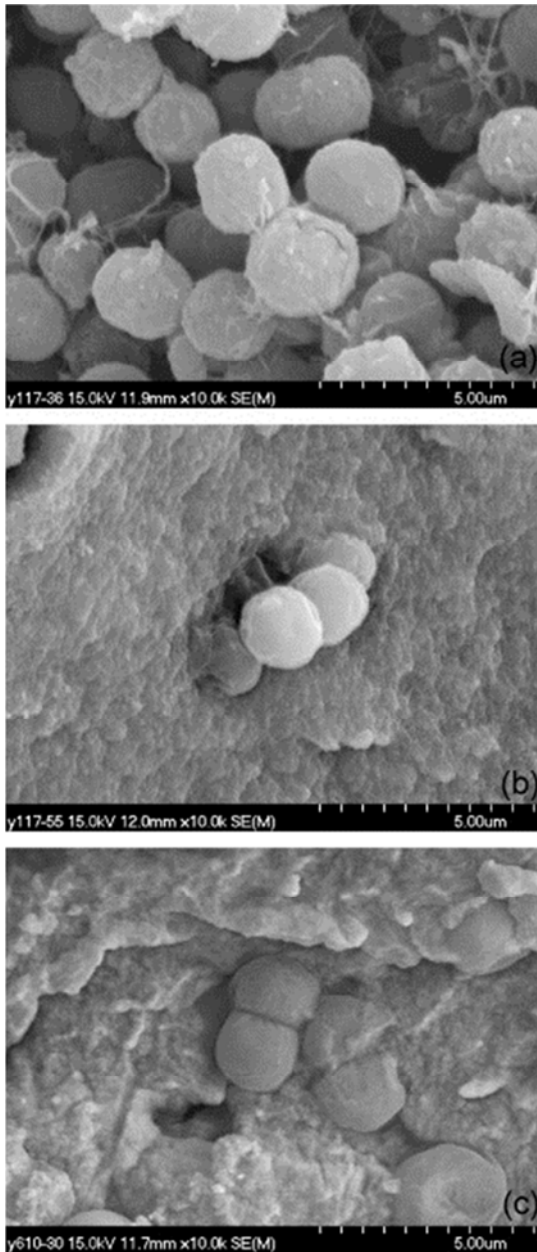


Figure 6. SEM images of fresh algae cells (a), algae cells treated by ECF for 60 min without Cl⁻ (b) and that with 1.0 mM of initial Cl⁻ (c) under the following conditions: current density, 2 mA/cm²; initial cell density, 1–1.5 × 10⁹ cells/L; initial pH, 7.0±0.2; temperature, 20±2°C [90].

The performant usage of ECF for water treatment is a function of working factors (i.e. electrode material, current density and temperature) and solution features like pH and existing ions. As an illustration, diverse contaminant

materials (oil, heavy metals, etc.) may be eliminated thanks to the useful role of chloride ions [90, 96]. Its significance was as well established in microalgae harvesting due to the production of active chlorine, which diminishes zeta potential and increases the liberation of metal ions. Therefore, for usual reasons, the existence of Cl⁻ in natural water bodies should not be overlooked [12].

Employing ECF has as well been proved to be more efficient when a sedimentation transition stage between ECF process and the microalgae flocs elimination is inserted, once microalgae suspension continues unstable following its elimination. Such destabilization can be more rapid with more elevated current densities. Nevertheless, it will as well conduct to more elevated liberation of metal ions from the sacrificial anode and more increased power consumption. The augmentation of turbulence and diminution of initial pH may elevate the performance of ECF also if aluminum is employed as anode [12, 97].

One more good technique for the harvesting of microalgae with no necessity for the injection of chemical agents is the electrophoresis. In this similar process to EC, an EF is imposed and the dispersed particles displace relative to a fluid. As the microalgae possess a net negative charge and their water-based development media contain monovalent sodium (Na⁺) and potassium (K⁺) ions, bacteria could displace in the direction of the anode if submitted to a DC EF [57]. This would lead to microorganisms' concentration since the negatively charged algae cells displace to the anode where they lose their electric charges inducing agglomeration and the generation of flocs [12, 98]. In such a method, the usage of a non-sacrificial anode would avoid the problem of metal ions pollution.

6. Conclusions

The main points drawn from this work may be given as:

1. Electrotechnologies cover remarkable ecological and treating benefits related to elevated energetic performances and less intensive utilization of non-renewable energy and natural resources.
2. Such technologies give the possibility to be used in upstream and downstream procedures of microalgae development such as genetic transformation and extraction of valuable compounds. It is practicable to dislocate microalgae cellular matrices (through electroporation impact) and assist the liberation of crucial bioactive molecules via the utilization of elevated voltage pulses procedures. Such technologies may as well give the chance to merge electrical and thermal impacts (electroheating), assuring possibility to deactivate culture pollutants.
3. Nevertheless, the performant contribution and useful advantages of such methods will ultimately be made public if many impasses at the phase of basic and engineered science have been conquered, like the necessity of: (1) a better comprehension of how electricity-based methods may be designed to

encourage the wanted impact (like cell disruption) on varying microalgae strains; (2) performing illustrative projects at industrial degree desiring elevated grades of technology easiness that must comprise detailed economic analyses and life cycle assessment; and (3) placing electrotechnologies use together with a big scale biorefinery idea via a merged and simplified planning of formation and valorization of all bulk constituents.

4. Methods setup on the usage of EFs are extremely encouraging for niche downstream/upstream utilizations, substituting or at least harmonizing with well-set technologies. Nevertheless, prosperous industrial application of such processes will be a function of a decisive interrogation – how much may they decrease global formation costs? Moreover, such methods can possess considerable action in controlling many of the restrictions of the technology, therefore augmenting cost-effectiveness of a selected microalgae biomass investment desiring the global formation method via, as an illustration, allowing the utilization of a biorefinery strategy.
5. As an electrotechnology, EC remains a viable process usable in microalgal biotechnology even if it is based on the partial or complete destruction of the cells. However, more researches should be planned in the perspective of a large industrial usage of this electrochemical technique.

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