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Application of HVED and other alternative technologies for microbial cell lysis and extraction of biocompounds

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Abstract

Microbial synthesis offers an interesting alternative to chemical synthesis and the means to industrially produce molecules whose production using synthetic means has yet to prove economically viable. Microbial compounds can either be synthesized intracellularly or extracellularly, and the intracellular ones can be challenging to recover given that the effectiveness and efficacy of cell disruption techniques are contingent upon numerous attributes of the microorganism's cell and those of the molecule to be recovered. High voltage electrical discharges or HVEDs are very commonly utilized to recover high-value compounds from plant matter and thus valorize biomass which would have otherwise provided little economic value. An increasing body of work has contributed to concretizing HVEDs' utility at disrupting microbial cells and expanding the technique's range of applicability.

Keywords: Cell lysis, cell disruption, high voltage electrical discharges, high pressure homogenization, bead milling, ultrasonication, pulsed electric fields, microwaves, microbial compounds.

I. Introduction

Microbial organisms can either naturally or by virtue of genetic engineering produce complex molecules with interesting applications in a variety of sectors. The use of microorganisms has

multifarious benefits, most notable of which being the large-scale production of molecules with hitherto no industrially viable means of synthesis or the sustainable production of environmentally friendly alternatives to unethically produced compounds (Paillière-Jiménez et al., 2020). While this description could position microbial synthesis below chemical synthesis in terms of overall yield, it does not detract from the significant utility of this modality as scientific literature abounds with an ever-broadening body of evidence as to the method's effectiveness, versatility, and economic value (Abdel-Aziz et al., 2017). Molecules of interest can either be accumulated intracellularly or secreted in the culture medium, with either of these options being highly dependent on individual attributes of the production chassis. Compound retrieval and isolation processes will consequently vary from one production chassis to the other, with intracellularly accumulated compounds requiring the use of cell disruption techniques to overcome the mechanical resistance of the cell wall and effectively, efficiently, as well as reliably extract the targeted high-value compounds (Otto et al., 2012). Depending on the characteristics of these molecules, those of the cell shape, and those of the microorganism's cell wall composition which factor into technique selection decisions (Alhattab et al., 2019; Kuboi et al., 1995), precautions must be taken to minimize molecule deterioration which may be incurred by substantial shear stress and sharp temperature increases for example by determining extraction parameters which reconcile both the retention of the compound's chemical integrity and its consummate recovery (Seo et al., 2013). This compromise is the most likely to ultimately culminate in an industrially viable extraction process (Nemer et al., 2021).

The composition of microorganisms' cell walls can differ considerably, resulting in some having greater mechanical or chemical resistance than others, thus necessitating the use of different cell disruption technologies or different sequential combinations of these technologies, provided of course that the risk of compound degradation is accounted for and mitigated. Given the considerable variability of cell wall compositions and shapes even among microorganisms of the same genus (Rast et al., 2017), general conclusions as to the aptitude of specific technologies at disrupting the cell walls of broad microorganism categories cannot be drawn (Yap et al., 2016). Indeed, this constricting specificity is further compounded by an array of remarkably specific variables such as microorganism harvesting, conditioning, and storage modalities which must also be accounted for as a result of their reasonably high impact on the organisms' pre-processing cell wall state (Bernaerts et al., 2019). The composition of the cell wall may also incur some mild complications associated with process diagnostics and evaluation. Bearing in mind that diffusivity (Safi et al., 2018) and thermal degradation (Alhattab

et al., 2019) can sometimes render target compound quantification an unreliable indicator of process effectiveness, additional data as to process effectiveness should be gathered by way of scanning electron microscope (SEM) imagery. This method can be utilized to investigate alterations of cell morphology and determine the extent to which they were adequately disrupted. However, this modality may be insufficient to draw reliable conclusions given that some cell morphologies, which depend in no negligible part on cell wall composition, may be misleading (Bernaerts et al., 2019). As such, these analyses could well be supplemented with fluorescence microscopy and a fitting staining technique. Given the multitude of implicated variables pertaining to both the cell disruption process and the microorganism's cell wall, conclusions as to cell disruption technique adequacy are usually scrupulous, specific, and characterized by a peculiarly limited scope.

The efficacy of a cell disruption technique is predicated on the exhaustive characterization of the processed product, yielding data from which conclusions can be drawn to make congruent modifications to the modus operandi and fine-tune the process parameters which also account for power consumption. Having acknowledged the drawback of restrictive specificity, this chapter addresses cell disruption techniques commonly utilized in a variety of sectors in addition to emerging techniques with appreciable potential. In particular, this chapter focalizes on high voltage electrical discharges or HVEDs which have gained traction as a nonthermal means of extracting high-value compounds from plant matter and microorganisms (Banožić et al., 2021a; Deng et al., 2018; Li et al., 2019; Liu et al., 2016a; Nutrizio et al., 2020; Roselló-Soto et al., 2015a).

II. High voltage electrical discharges technology

Electrical discharge technology has been recently developed and used to enhance the extraction of biocompounds from multiple raw materials (Boussetta and Vorobiev, 2014). High-voltage electrical discharges (HVED) technology involves the application of high voltages through an aqueous solution. Therefore, it is capable of extracting different intracellular compounds by damaging the structure of the treated raw material, such as microbial cells or plant food materials (Sridhar et al., 2021). HVED was previously used to enhance the extraction of polyphenols from white grape pomace, as well as solutes from soybeans and potatoes (Roselló-Soto et al., 2015b). Additionally, HVED was successfully used to disrupt yeast cells, such as *Saccharomyces cerevisiae*, in order to extract their intracellular proteins. In fact, it was reported that using HVED had no negative effect on the quality of proteins extracted from the microbial

cells (Liu et al., 2016b). Evidence suggests that HVED treatment mechanically damages the membranes of yeast cells and leads to the disintegration of their cell walls (Shynkaryk et al., 2009).

The principle of HVED extraction is based on the electric discharges generated directly into water through the plasma channel formed by the high voltage electrical discharges between two submerged electrodes (Figure 1). This electrohydraulic discharge (an electric discharge generated directly into water) leads to disturbance in the medium, generates strong dynamic shock waves, and induces chemical and physical reactions in water (Boussetta and Vorobiev, 2014). In fact, during the formation of the plasma channel in the vessel, an intense shock wave, between 5 and 20 kilobar, is generated due to the rapidly expanding plasma channel (Boussetta et al., 2012). This shock wave might induce free-radical and pyrolytic reactions indirectly through hydraulic cavitation. Rarefaction wave producing cavitation follows the previous pressure shock wave. The collapsing cavitations create strong secondary shockwaves with a very short duration (Boussetta et al., 2012). Hence, the electrical discharge leads during water photodissociation to the generation of hot, localized plasmas that emit high-intensity ultraviolet light, shock waves, and produce free radicals, including hydrogen peroxide, hydroxyl radicals, and oxygen radical ions (Banožić et al., 2021b). All of the aforementioned processes lead to the mechanical rupture of cell walls and membranes and accelerate the release of intracellular compounds into the solvent (Grimi et al., 2014).

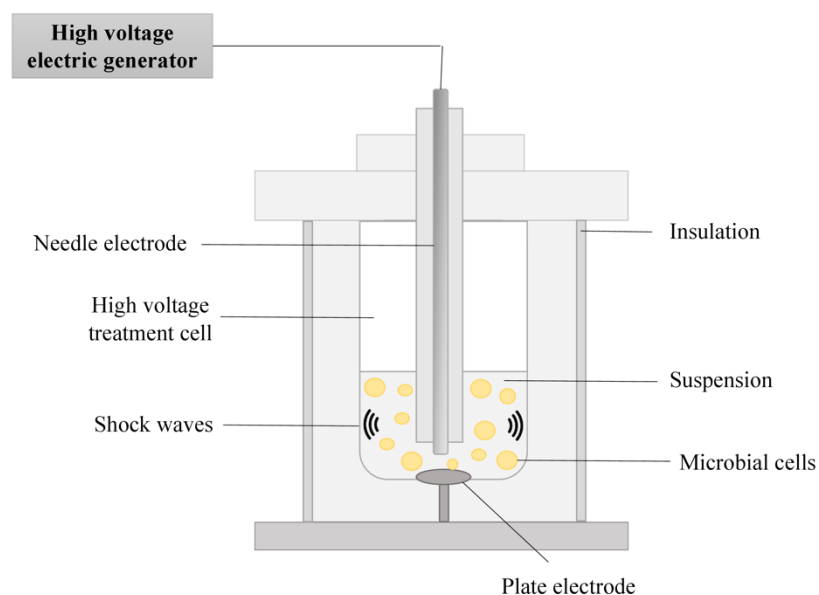


Figure 1. High voltage electrical discharges apparatus

It is noteworthy to mention that HVED was demonstrated to be highly effective at extracting high molecular weight constituents, such as proteins, from electrically resistant microbial strains (Grimi et al., 2014). Additionally, HVED proved to be more effective, in terms of energy input and effective treatment time, at the extraction of phenolic compounds and proteins, compared to ultrasound and pulsed electric fields (PEF) (Roselló-Soto et al., 2015b). HVED is a non-thermal and green extraction technique. It enhances the extraction of bioactive compounds and increases the recovery yield while using low treatment energy input. It also shortens the treatment time (few milliseconds), decreases the applied temperature, and improves the quality of the product. In effect, HVED does not require the use of organic solvents (Banožić et al., 2021b; Liu et al., 2011).

The specific energy input of the treatment E (kJ/kg) can be calculated using:

$$E = \sum_{i=1}^n \frac{W_{i(\text{HVED})}}{m}$$

where $W_{i(\text{HVED})}$ is pulse energy (kJ/pulse), n is the number of pulses, and m is the product mass (kg). $W_{(\text{HVED})}$ can be calculated using:

$$W_{(\text{HVED})} = \int_0^t V \cdot I \, dt$$

Where V is the voltage (V) and I is the current strength applied (A) (Sridhar et al., 2021).

III. Other cell disruption technologies

III. 1. High pressure homogenization

High pressure homogenization (HPH) is a mechanical process widely used to disrupt microorganisms in pharmaceutical and biotechnological industries. The homogenizer mainly consists of a displacement pump, valve seat, homogenizing valve and impact ring (Figure 2). The cell suspension is circulated by the pump under high pressure through a narrow gap also called a homogenizing nozzle or a high-pressure valve, located between the valve and the valve seat. As the suspension exits the unit, the flow velocity increases rapidly and the pressure decreases to atmospheric pressure (Yap et al., 2015). Equipment is called “high pressure homogenizer” when the pressure reaches 150-200 MPa and “ultra-high pressure homogenizer” when the pressure goes up to 350-400 MPa (Comuzzo and Calligaris, 2019). The mechanisms of cell disruption induced by HPH are not yet fully understood. However, the combined effects of fluid shear stress resulting from its circulation through the nozzle, the interaction of the fluid with the valve and the impact ring, and the pressure difference between the nozzle and the

outside environment could lead to cell membrane rupture (Koubaa et al., 2020). The operating pressure and the number of passes through the valve are the main parameters to adjust depending on the type of microbial cells (Gomes et al., 2020).

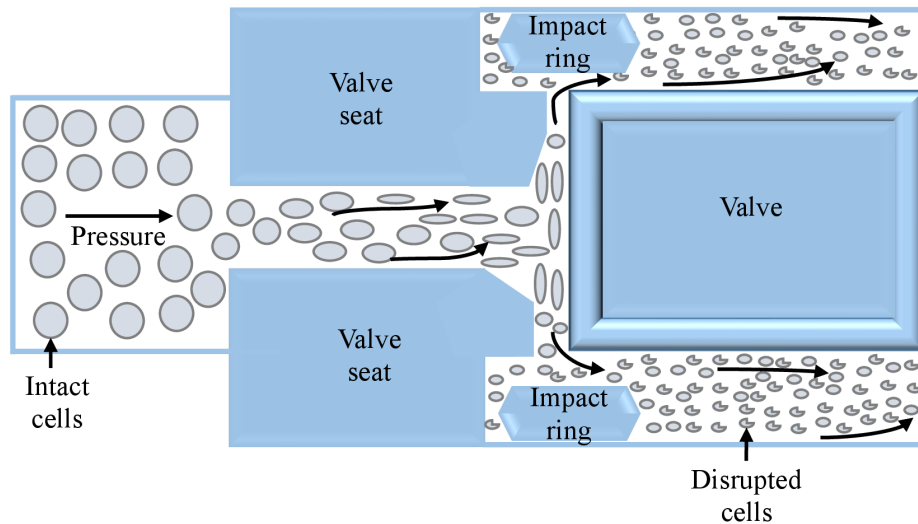


Figure 2. Schematic representation of high-pressure homogenizer. Adapted from (Gomes et al., 2020).

III. 2. Bead milling

Bead milling is a mechanical technique that has been successfully applied at laboratory and industrial scale for cell disruption and downstream processing of intracellular compounds. In general, a bead miller is composed of agitator disks of varied design (concentric or eccentric) connected to a rotating shaft inside a grinding chamber (Günerken et al., 2015) (Figure 3). During the operation, glass or stainless-steel beads are placed inside the chamber. Cell-bead collisions create shear forces and favor the grinding of the cells between the rolling beads which result in cell wall disruption (Zainuddin et al., 2021). The efficiency of cell disruption depends on the size and bead load, temperature, agitation speed, and treatment time. Other factors including the type of microorganisms and their concentration in the culture medium should be considered. For example, glass-beads with a diameter of 0.1 mm are used for the disruption of bacteria cells, while glass-beads with 0.3-0.4 mm and 0.5-1.25 mm, are more efficient for the disruption of microalgae and yeasts or fungi, respectively (Gomes et al., 2020).

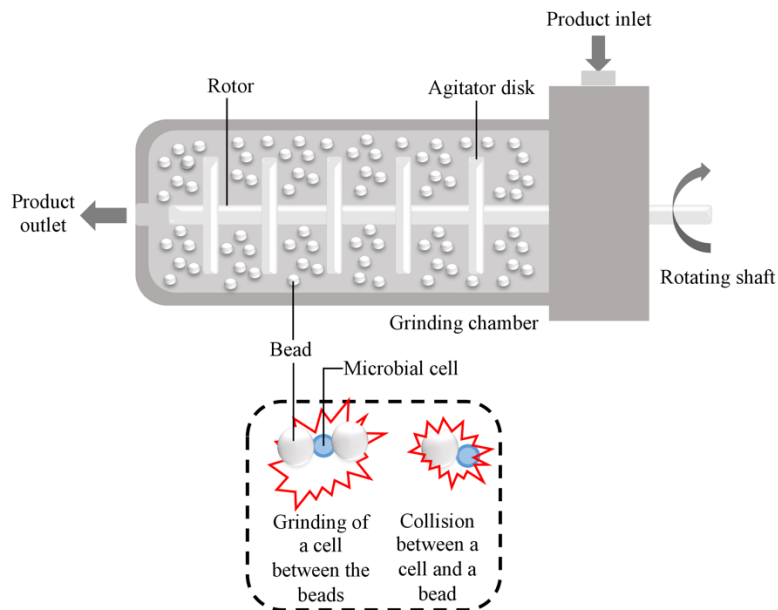


Figure 3. Schematic representation of cell disruption by bead milling. Adapted from (Ishii, 2018).

III. 3. Ultrasonication

Ultrasound is another mechanical cell disruption technique based on the “cavitation” phenomenon. It has multiple applications in the food industry including freezing, cutting and drying because of its efficiency and easy use and implementation. In bioprocess, ultrasound is used for cell lysis. Ultrasound waves propagate in a liquid medium and create alternations of low-pressure (rarefaction) and high-pressure (compression) cycles. Micro bubbles of gases are formed during low-pressure cycles, expand, and collapse during high-pressure cycles, producing intense shock waves (cavitation) (Pingret et al., 2013). The cavitation phenomenon induces a local increase in temperature and pressure which may damage cell walls. In addition, the thermolysis of water around the bubbles results in the production of highly reactive free radicals (D’Hondt et al., 2017). A probe-type sonicator is mostly used in the laboratory scale (Figure 4). An ultrasonic generator is bonded to a titanium probe which is immersed in a liquid phase to deliver the ultrasonic intensity through a small surface (only the tip of the probe). There are numerous designs of probes with different lengths, diameters and tip geometries. Several parameters such as ultrasound power and frequency, ultrasonic intensity, liquid temperature, cell size, and treatment time may affect cell disruption efficiency (Chemat et al., 2017; Yusaf, 2015).

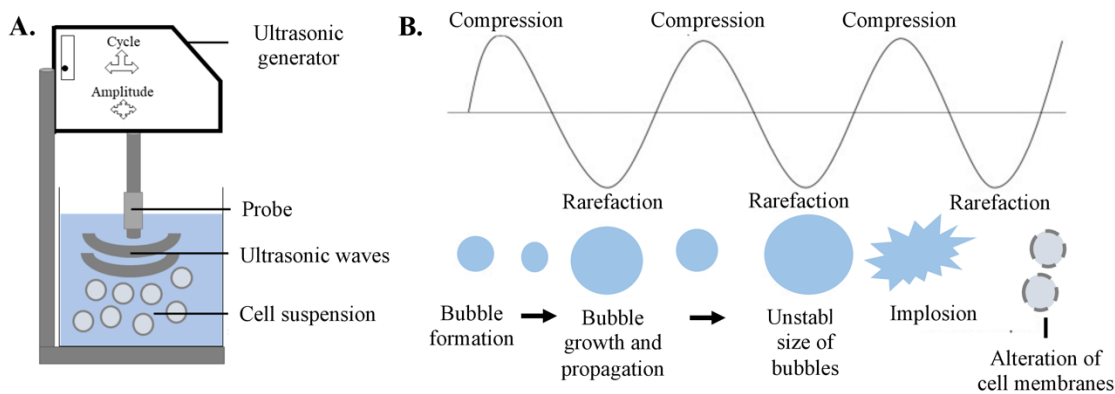


Figure 4. Cell disruption using ultrasound. **A.** Laboratory probe-type sonicator. **B.** Mechanism of action of ultrasounds through the cavitation phenomenon.

III. 4. Pulsed electric fields (PEF)

PEF is a non-thermal technology, efficient for microbial cell lysis due to the electroporation of cell membranes. PEF system is mainly composed of a treatment chamber connected to a high voltage pulse generator (Figure 5). Cell suspension is placed in the chamber between two plane electrodes and pulses with electric field strengths varying from $100\text{-}300\text{ V}\cdot\text{cm}^{-1}$ and $20\text{-}80\text{ kV}\cdot\text{cm}^{-1}$ are applied for short periods of time (ns to ms). A transmembrane potential (u_m) is therefore created between the two sides of the cell membranes. When this difference in potential reaches a specific critical value, pores are formed in the cell membrane (electroporation) (Koubaa et al., 2018). Depending on the PEF treatment conditions such as electric field strength, number of pulses and exposure time, the electroporation could be reversible with pore resealing (lasting from seconds to hours) or irreversible with complete damage of the membrane (Barba et al., 2015).

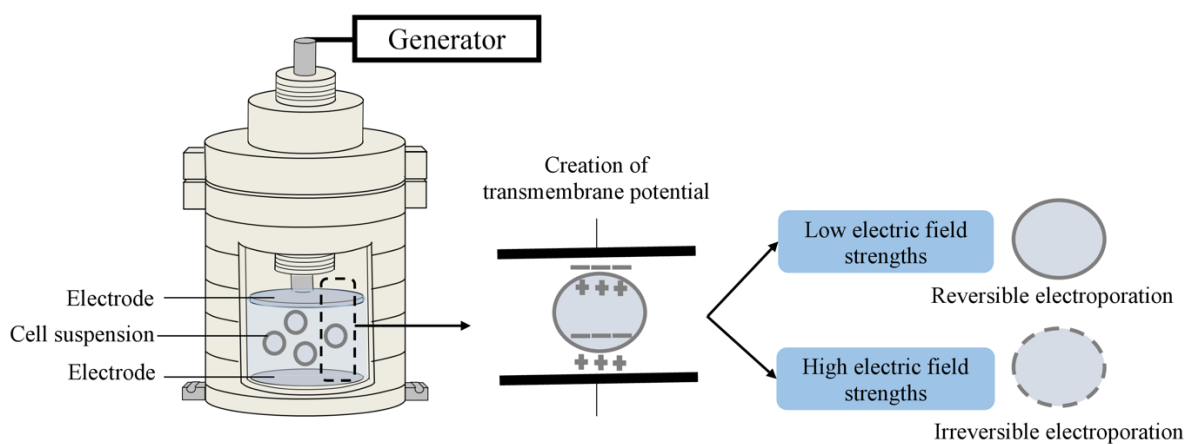


Figure 5. Cell disruption by pulsed electric fields.

III. 5. Microwaves

Microwaves are used for cell disruption and recovery of intracellular compounds. When a suspension is treated with microwaves, the electromagnetic energy (frequency range of 300 MHz-300 GHz) is converted to heat following dipole rotation and ionic conduction (Barba et al., 2016) (Figure 6). Microwaves interact with polar molecules and cause a sudden increase of temperature and pressure as a result of frictional forces from inter- and intramolecular movements (Günerken et al., 2015). The local heat and pressure generated by microwaves induces damage to the cell wall and accelerates the release of intracellular compounds into the surrounding extraction solvents (Koubaa et al., 2020). The efficiency of microwaves is mainly affected by the applied power and the treatment time.

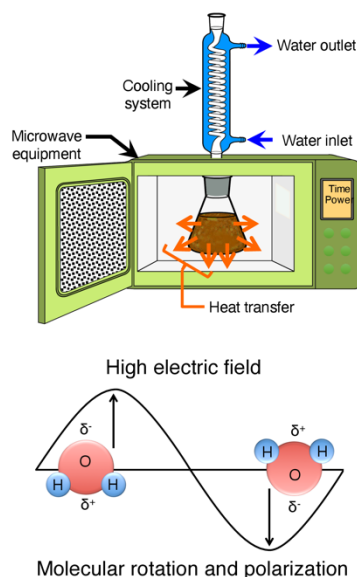


Figure 6. Microwaves equipment used at laboratory scale showing the molecular rotation mechanism. Reprinted with permission from (Barba et al., 2016).

IV. Extraction of valuable compounds using emerging technologies

IV.1. Extraction of valuable compounds from yeasts and fungi

The effect of HVED was studied on the yeast *Saccharomyces cerevisiae* (Loginov et al., 2010; Shynkaryk et al., 2009). For instance, Shynkaryk et al., (2009) treated a yeast suspension with 40 kV discharges, generated between electrodes with a needle-plate geometry separated by 10 mm. Successive pulses (up to 150) of 1-2 μ s were applied with a frequency of 0.5 Hz. This technique was compared to PEF and HPH. For PEF, the suspension of *S. cerevisiae* was subjected to a continuous treatment (250 mL.min⁻¹) at 3-10 kV.cm⁻¹. It consisted of successive trains (up to 1,000) of 200 pulses of 100 μ s with a repetition rate of 200 Hz. For HPH, the yeast

suspension was subjected to successive passes (up to 20) at 30 to 200 MPa with a flow rate of 10 L.h⁻¹. The significant release of ions after these different treatments was evaluated through the disintegration index Z . To achieve a high level of membrane disintegration, corresponding to $Z > 0.8$, around 1,000 trains of PEF at 10 kV.cm⁻¹ were needed. It required more than 100 pulses at 40 kV.cm⁻¹ for HVED and 5 passes at 100 MPa for HPH. Moreover, in this work, the authors highlighted a synergistic effect when combining HVED and HPH. Indeed, a pretreatment of yeast cells with HVED allowed to reduce pressure or number of passes to reach a given disintegration level. For example, to achieve $Z > 0.8$, only 1 pass at 100 MPa was necessary after HVED pretreatment at 40 kV.cm⁻¹ (leading to $Z_i = 0.15 \pm 0.05$).

More recently, Ganeva et al., (2020) studied the impact of PEF on the yeast *S. cerevisiae* for the extraction of various bioactive compounds. The optimum parameters found were a continuous process of a suspension containing 66 gDCW.L⁻¹ at a flow rate of 27 mL.min⁻¹, with 19 pulses of 0.5 ms at a field strength of 3 kV.cm⁻¹. After 16 h of incubation in an alkaline buffer, they were able to extract 87.8±3% of the total proteins obtained with the glass bead disruption method. These conditions were also favorable to the release in water of 90-95% of the total free amino acids, 78±4.6% of the total antioxidant compounds, 78±6% of total glutathione, 41% of total phenolic content, and 92% of the low molecular UV absorbing substances.

Recently, the yeast *Yarrowia lipolytica* has gained interest for the specific extraction of oils using emerging technologies (Drévillon et al., 2019, 2018; Imatoukene et al., 2020). For instance, Drévillon et al., (2019) investigated several techniques for the disruption of *Y. lipolytica* cells including mechanical expression assisted or not with moderate PEF, high PEF alone, HVED, ultrasounds, and HPH. For the HVED treatment, a suspension of yeasts containing 15% dry cell weight (DCW) was placed in a reactor chamber with a pair of needle-plate electrodes. The cells were subjected to 500 pulses of 10 μs at 40 kV.cm⁻¹. The authors were able to extract 31.7 ± 6.5 % of total lipids compared to 19.8 ± 0.5% for the untreated suspension. However, they also noticed a significant modification in the fatty acids profile of the oil obtained with this technique. HVED are accompanied by physical and chemical effects such as the formation of oxidizing species, which could have degraded fatty acids, especially the unsaturated ones. Among the different pretreatments investigated, HPH was the most interesting, allowing to recover 83.9 ± 4.8 % of total oil. HPH was further explored for the extraction of valuables from *Y. lipolytica*. It was shown that only 5 passes at 150 MPa were

sufficient to release large amounts of ions, proteins, nucleic acids, and lipids (Dréwillon et al., 2018).

In another recent work, Imatoukene et al., (2020) coupled the HPH treatment with bead milling for a better lipid extraction from *Y. lipolytica*. They demonstrated that the combination was more efficient than bead milling alone. It even allowed getting high recovery yield when applying the extraction directly on wet biomass or when using green solvents. For instance, the lipid yield increased from 9.0% with bead milling alone applied on wet cells to 57.8% when adding an HPH pretreatment. Moreover, this article studied in more details bead milling and its operating parameters. The authors established that a larger bead size or a longer processing time resulted in a higher lipid yield.

Ultrasonication was also applied for the extraction of oil from other oleaginous yeasts such as *Rhodospiridium* species (Meullemiestre et al., 2017; Patel et al., 2019). The effect of sonication alone or coupled with temperature, pressure or both (manothermosonication) was studied on *Rhodospiridium toruloides* (Meullemiestre et al., 2017). Manothermosonication gave the best results with 26.94 g.100gDCW⁻¹ when used as a pretreatment and 29.74 g.100gDCW⁻¹ when used directly with an extracting solvent. With SEM, the authors noticed damage on the cell surface. The degree of disruption was in agreement with the lipid yields obtained with the different techniques. Patel et al., (2019) combined a rapid ultrasonication operation (2 min at 40 kHz) followed by microwaves for the treatment of *Rhodotorula kratochvilovae*. The optimization of microwave operating parameters (temperature, duration, and ratio solvent/biomass) allowed them to recover a high amount of lipids, 6.54 g.L⁻¹ representing 70.86% of the DCW.

Yeast cells are also an interesting resource for pigments such as carotenoids. Liu et al., (2019) investigated chemical, enzymatic, and physical methods for the recovery of carotenoids from *Sporidiobolus pararoseus*. HPH was found to be the best treatment as it provided high amounts of pigments while avoiding their oxidation. Furthermore, the operating parameters were optimized and found to be 80 MPa during 3 passes with a cell concentration of 8%. Under these conditions, the authors recovered 82.5% of the total carotenoids.

The enhancement of carotenoid extraction with PEF was explored by Martínez et al., (2018). They treated a yeast suspension at 10 gDCW.mL⁻¹ in a continuous chamber with a flow rate of 4 L.h⁻¹. With an electric field of 15 kV.cm⁻¹ during 150 μs of total treatment time, representing 37.12 kJ.kg⁻¹, more than 90% of the cells were irreversibly electroporated. The authors then

optimized the downstream process conditions to better extract the pigments. After a 1-day incubation in a citrate-phosphate buffer at 25°C and pH = 8 followed by an ethanolic extraction for 1 h, they were able to recover 375 µg.gDCW⁻¹ of carotenoids.

IV. 2. Extraction of valuable compounds from bacteria

HVED were applied to the cyanobacteria *Microcystis aeruginosa* (Zhang et al., 2017). A batch of 250 mL of a suspension of *M. aeruginosa* was treated between a pair of needle electrodes by 3, 4, or 5 kV electrical discharges for the same duration. After 5 days of incubation post-treatment, the optical density of the suspension, corresponding to the cell concentration, had decreased by 73% when exposed to 4 or 5 kV, and 36% when exposed to 3 kV. It was demonstrated that electrical discharges inactivated the cyanobacteria and led to cell death. This observation was confirmed by the reduction of chlorophyll-a and microcystin-LR contents. For example, after a treatment at 5 kV, a decrease of 90% and 100% for chlorophyll-a and microcystin-LR, respectively, was noted.

Geada et al., (2019) also studied the disintegration of *M. aeruginosa* by physical means. They optimized the processing parameters of bead milling, microwaves, freeze-thawing, high-speed homogenization, and sonication. Among these technologies, ultrasounds (40 kHz, 10 min), assisted or not by freeze-thawing (3 cycles at -20°C), gave the best results concerning the reduction of cells' viability and the release of organic matter and microcystins.

Among cyanobacteria, *Arthrospira platensis* and *Arthrospira maxima*, known as spirulina, were extensively studied for the extraction of phycocyanin using alternative technologies (Carullo et al., 2021, 2020; Jaeschke et al., 2019; Käferböck et al., 2020; Li et al., 2020).

Li et al., (2020) treated a suspension of *spirulina* at 6%DCW using different technologies: PEF (20, 25, or 30 kV during 10, 50, or 200 pulses), high-pressure processing (HPP) (3.5 min at 50, 100, 200, 400, or 600 MPa) or ultrasonication (2, 4, or 6 min at 20, 30, or 40% amplitude). PEF and HPP treatments did not improve the extraction yield of phycocyanin and even denatured the pigment when the most severe conditions were applied (30 kV, 200 pulses or P ≥ 200 MPa, respectively). An increase in the ultrasonication time or amplitude enhanced the phycocyanin yield. However, this technique also generated many cell fragments and was not highly selective with the co-extraction of other pigments.

Conversely, in several studies, PEF technology was successfully applied on *spirulina* for extracting substantial amounts of phycocyanin with high purity (Carullo et al., 2021, 2020; Jaeschke et al., 2019; Käferböck et al., 2020). Jaeschke et al., (2019) treated a suspension of *A.*

platensis by PEF at 40 kV.cm⁻¹ delivering a specific energy of 112 J.mL⁻¹. The amounts of phycocyanin and proteins were similar to those obtained with bead milling. Nevertheless, electroporation allowed a more selective extraction of water-soluble components compared to bead milling, and the extracts had a better antioxidant activity. Käferböck et al., (2020) obtained similar results with *A. maxima*. With a combination of freeze-thawing and PEF (specific energy of 100 kJ.kg⁻¹, but only 25 kV.cm⁻¹), they obtained high yields of phycocyanin and proteins, representing respectively 90% and 72% of the bead milling control sample.

Carullo et al., (2021, 2020) compared PEF treatments to HPH for the extraction of valuable compounds from *spirulina*. PEF treatment alone did not provide interesting yields. However, PEF (20 kV.cm⁻¹ and 100 kJ.kg⁻¹) combined with high shear homogenization (20,000 rpm, 1 min) released great amounts of proteins, carbohydrates, and phycocyanin corresponding respectively to 67.2, 87.7, and 88.6% of the HPH control. Likewise, they highlighted a synergistic effect between PEF (same conditions) and temperature. At 35°C, they were able to extract 34.4, 73.1, and 72.5% of proteins, carbohydrates, and phycocyanin compared to HPH reference.

Another type of bacteria, *E. coli*, was studied by Haberl Meglič et al., (2020) for green fluorescent protein (GFP) extraction using PEF, ultrasonication or bead milling. For PEF treatment, they investigated different combinations of parameters: batch or continuous treatment, variable electric field strength, frequency, number, and duration of pulses. For sonication, a suspension of bacteria was treated with ultrasounds at 90 W, 25 Hz during 3 x 20 s. Finally, for bead milling, they mixed glass beads and an *E. coli* suspension with a 1:1 ratio and homogenized them at 2850 rpm for 5 min. Ultrasonication and bead milling gave the highest total protein and GFP yields. These two techniques were thus not selective for GFP and they also generated many fragments which could hinder downstream purification. The best PEF treatment consisted of 8 pulses of 1 ms at 5 kV.cm⁻¹ with a frequency of 1 Hz, corresponding to 37 kJ.L⁻¹. Indeed, it gave the highest GFP yield with the lowest amounts of contaminants and fragments.

IV. 3. Extraction of valuable compounds from microalgae

Eukaryotic microalgae are widely studied for the production and extraction of valuable compounds such as pigments or lipids for biodiesel. Zhang et al., (2020a, 2020b) investigated the effect of HVED (40 kV.cm⁻¹), PEF (20 kV.cm⁻¹), and ultrasounds (200 W) on three different microalgae, namely *Nannochloropsis* sp., *Phaeodactylum tricornutum*, and *Parachlorella kessleri*, for the recovery of proteins, carbohydrates, and chlorophylls. Different treatment times

were delivered, corresponding to different specific energy consumption. PEF technology was not efficient for enhancing the extraction, regardless of the microorganism and the extracted compound. HVED was the most effective techniques for the recovery of carbohydrates. For instance, the authors achieved a yield of $43.7 \pm 1.6\%$ with *Nannochloropsis* sp. (Zhang et al., 2020c). Ultrasounds gave the best results for proteins but, overall, the amounts of proteins were low despite the pretreatments. Proteins are large molecules located in different cell compartments; therefore, it is possible that stronger techniques are needed for an efficient recovery. Finally, for pigments, ultrasonication was the most efficient treatment allowing to recover 18 to 40% of total chlorophylls. The authors also highlighted that the differences observed between the three species studied could be linked to the cell size and morphology, to the cell wall composition, or to the location of compounds inside the cell.

For each of these microalgae species, the same team of researchers further explored HVED, other techniques, and combinations of techniques to improve the recovery of various bioactive molecules (Zhang et al., 2021, 2020d, 2020b, 2019).

HPH allowed to recover higher yields of ions, proteins, carbohydrates, and pigments than ultrasounds (Zhang et al., 2019). However, because of its harshness, it was not selective and generated extended cell damage leading to cell fragments. The combination of these technologies led to various effects depending on the extracted molecule. The best compromise between high extraction yield and limited energy consumption was obtained with the ultrasonication of a concentrated suspension at 10%DCW followed by the HPH of the corresponding diluted suspension at 1%DCW.

HPH was also compared to HVED for the extraction of valuable compounds in a multistep procedure (Zhang et al., 2020b). HPH was demonstrated to be more efficient for the recovery of proteins, carbohydrates, and pigments in water. However, HVED proved a better efficiency for the release of ions in water and for solvent extraction of pigments and lipids.

Furthermore, the combination of PEF or HVED with HPH improved the recovery of carbohydrates but not for proteins (Zhang et al., 2021). It has been demonstrated that applying HVED on a concentrated suspension of microalgae followed by HPH allowed to obtain high yields of carbohydrates and proteins while limiting energy consumption.

Zocher et al., (2016) studied numerous techniques for the extraction of proteins and pigments from *Chlorella vulgaris*. Especially, they investigated different methods (ultrasound, HPH, and microwaves) and various electrical techniques including PEF and different plasma generation

sources (corona discharges, DC-plasma jet, volume dielectric barrier discharges, pin-to-liquid discharges, and spark discharges). Ultrasonication and HPH barely extracted the targeted molecules. This could be due to the rigid cell walls of *C. vulgaris* and to its small round shape. Microwaves released the highest amount of proteins (> 5% of dry weight) and a significant quantity of pigments (for instance, 6.836 to 15.350 mg.gDCW⁻¹ of chlorophyll a). However, it was also demonstrated that they could degrade sensitive molecules, hence a modification of the color extract. Among the different technologies; PEF, plasma jet, and volume dielectric barrier discharges were inefficient for extracting both proteins and pigments. Corona discharges and pin-to-liquid discharges slightly increased the pigment yield but were not efficient to extract proteins. The authors suggested a mechanism of electroporation during the treatment to explain these observations. Finally, spark discharges gave interesting recovery yields, as high as those obtained with microwaves but without loss in color. The authors demonstrated that these levels of extracted compounds are the result of a physical action more than a chemical effect.

Zocher et al., (2019) further compared microwaves and spark discharges pretreatments. With proteomic analysis, they identified the extractable proteins from *C. vulgaris* and determined the differences between these two techniques. Spark discharges seemed to be more suitable for the recovery of thermosensitive components. Indeed, they allowed an up-regulation of chlorophyll a-b binding proteins, photosystem-related proteins, and histones in the supernatant in comparison with microwaves. However, spark discharges could also induce chemical degradation of proteins, mostly due to oxidation.

The production and extraction of pigments from microalgae were of prime interest for several studies (Martínez et al., 2019a, 2019b; Sommer et al., 2021). Sommer et al., (2021) explored various physical treatments for the extraction of phycocyanin from *Cyanidium caldarium*. Freeze-thawing, PEF, and intermittent ultrasounds proved to be inefficient. Continuous ultrasounds gave the highest recovery yield (53 mg.gDCW⁻¹) but the recovery decreased to 13 mg.gDCW⁻¹ after purification, indicating an important contamination of the raw extract. Spark discharges allowed to recover only 4 mg.gDCW⁻¹ but with a high purity and the least peptide modifications.

As demonstrated by the previously reviewed scientific papers, PEF gave mixed results on the extraction of valuable compounds from microalgae. Indeed, this treatment helped releasing interesting amounts of ions and carbohydrates but was very limited for proteins recovery, where HPH, bead milling, and enzymatic digestion turned out to be more efficient (Carullo et al., 2018; Lam et al., 2017; Safi et al., 2017). However, two recent studies highlighted the

importance of an incubation time following the PEF treatment (Martínez et al., 2019b, 2019a). Every pretreatment applied on *Haematococcus pluvialis* by (Martínez et al., 2019b) increased the pigment recovery yield, and PEF was the most efficient technique, though under specific conditions. Indeed, it needed a 6-hour incubation in ethanol to recover up to 96% of the total carotenoids. Authors argued an esterase activity to explain these observations. Likewise, PEF-treated *Porphyridium cruentum* were incubated in citrate-phosphate Mcllvaine buffer (Martínez et al., 2019a). After 24 h, it led to 30 mg.gDCW⁻¹ of β -phycoerythrin of the maximum 32 mg.gDCW⁻¹ obtained with bead milling. Again, a mechanism with enzyme activity enhanced by PEF was proposed.

The same observations were made for lipid recovery from *C. vulgaris* (Canelli et al., 2022). The authors compared several treatments, namely PEF (10 pulses of 5 μ s at 20 kV.cm⁻¹), enzymatic (mix of 3 hydrolases), a combination of both, and HPH (4 passes at 100 MPa). First, it was confirmed that an incubation time after PEF processing improved lipid bioaccessibility. For instance, 12 h at 25°C or 37°C or 48 h at 4°C allowed to reach a maximum of 18.7-20.9% of lipid bioaccessibility, against less than 10% with no incubation. Then, the combination of enzymes and PEF no further improved this result, and no synergistic effect was observed. Finally, HPH gave the highest lipid bioaccessibility (56.5 \pm 2.2%) but with degraded quality as their oxidative stability upon storage was low. On the contrary, PEF and enzyme treatment preserved the quality of lipid extracts.

Other techniques were explored for the recovery of lipids from microalgae, such as microwaves, ultrasounds, autoclaving, osmotic shock, and Soxhlet extraction (Ansari et al., 2018; Hurtado et al., 2018). Microwaves seemed to be a promising process as it gave interesting recovery yields in short treatment times. It also preserved the degradation of sensitive fatty acids such as unsaturated ones.

V. Conclusion

Cell disruption techniques vary in their effectiveness due to a considerable number of parameters including cell morphology, cell wall characteristics, chemical compound properties, diffusivity in the solvent, and the intracellular locus of the molecule of interest among others. These techniques could be utilized individually or sequentially within a molecule recovery process in such a way as to ensure industrial viability. The success of given configurations hinges on striking a balance between costs, time, as well as yields. HVED is gaining momentum as a cell disruption method used to recover microbial molecules of interest and the breadth of

its applicability is likely to increase. While it is not necessarily poised to replace counterparts which have already proven their efficacy in specific applications, it certainly contributes to increasing the ambit of extractive possibilities.

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