Integration of Pulsed Electric Fields in the Biorefinery Concept to Extract Microalgae Components of Interest for Food Industry

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Abbreviations

PEF	Pulsed electric field
HPH	High-pressure homogenization
HSH	High shear homogenization
DW	Dry weight
BDW	Biomass dry weight
SUS	Suspension

Introduction

"One of the greatest global challenges of the 21st century is to provide a growing world population with sustainable food, raw materials and energy in times of climate change." This is not only the strategy of a national German research programs according to *Bioeconomy 2030*, BMBF (2010), but also part of the next EU research and innovation investment program *Horizon Europe*.

Microalgae can help to tackle this important challenge as these autotrophic growing microorganisms are able to bind CO_2 and thereby to reduce greenhouse gas emission and, in addition, to produce valuable components such as proteins, carbohydrates, and lipids. Some algae species can produce up to 60% of their body weight in the form of triacylglycerols (Metzger and Largeau (2005)), which are lipids consisting of three long chains of fatty acids attached to a glycerol backbone. These lipids are similar to triacylglycerols found in large quantities in natural oil from oilseed crops that are suitable as biodiesel. An important feature of microalgae production is that they do not have to compete with other biomass alternatives but have the capacity to use water and land resources that are not considered for crop

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production. As a result, over the past three decades, algae have been widely discussed as an alternative source of biofuel that does not compete with the existing oilseed market.

Nowadays, an energy neutral production of microalgae biomass and their subsequent utilization for production of biofuel are unfeasible. This is paid, on one hand, to the high costs for microalgae cultivation (e.g., circulation and mixing) and, on the other hand, to the intensive downstream processing, which requires harvesting, drying, cell disruption, and finally purification of the product. These costs can be attributed up to 60% of the total production costs (Coons et al. (2014), Delrue et al. (2012), Molina Grima et al. (2003)). One solution to increase the efficiency is to use the residual biomass after obtaining high- and middle-value products (e.g., pigments, polysaccharides, or proteins) for biofuel production. This idea of splitting an educt into several products of different chemical composition and value is called biorefinery (Posten & Walter (2012)). The International Energy Agency (IEA) (2009) defines biorefiney as "the sustainable processing of biomass into a spectrum of marketable products and energy." Following this concept, a cascade processing of the microalgae has to be used in order to enable multiple component recovery, which improves the economics (Ruiz et al. (2016), Vanthoor-Koopmans et al. (2013), Wijffels et al. (2010)).

Valuable algal components are generally stored intracellularly either in organelles such as oil droplets or bound to membranes, which are enclosed by robust polysaccharide cell walls. Therefore, effective pre-treatment methods are required to break the cell envelopes, especially the cell wall, while the quality of the target components is not impaired. Conventional cell disruption technologies for component release, e.g., bead milling or high-pressure homogenization (HPH), can break this barrier but are energy-intensive and provide poor product quality since component fractions are either mixed or emulsified. Cell disruption methods must ensure that the products are not impaired in terms of quality and functionality by avoiding exposure to caustic agents and undesirable heating effects due to shear forces and high pressures. For this application, PEF treatment can be used as a mild and effective method of cell disruption, facilitating recovery of unaltered constituents at low energy costs (Vanthoor-Koopmans et al. (2013), Grimi et al. (2014)). PEF technology allows cascade processing for multiple component recovery, since it does not destroy cell shape and maintains gravimetrical biomass separability after single extraction step (Eing et al. (2013), Goettel et al. (2013), Kotnik et al. (2015), Silve (2018a, b), Jaeschke et al. (2019), Scherer et al. (2019), Akaberi et al. (2019, 2020)). In our approach, water-soluble proteins and carbohydrates are recovered first, followed by a solvent extraction of lipids, and all this in a wet process without the necessity to dry the biomass. This would contribute to significant energy savings in industrial-scale processes and also to a full valorization of the algae biomass.

In the last years, the focus of research is shifting toward using microalgal extracts as nutritional supplements, as fertilizer, or as raw material for cosmetic and pharmaceutical products (IGV Planttech (2019), Biorizon biotech (2020)). Therefore, this chapter focuses on the first stage of the biorefinery cascade: the PEF-assisted recovery of proteins and pigments of *C. vulgaris* and *A. platensis* as representatives for green algae and cyanobacteria, respectively. Improving the treatment procedure and

subsequent downstream processing of the biomass are other aspects that are discussed in this chapter. Using the examples of these two microorganisms, the requirements for upscaling this process to high biomass throughputs and the mechanisms of protein release are also discussed.

Cell Disintegration Methods

Conventional cell disruption technologies for component release, e.g., bead milling, HPH, high shear homogenization (HSH), ultra-sonication, and thermal and chemical treatment, can break the cell envelope (consisting of cell membrane and cell wall) and are therefore very efficient in recovering individual components. A drawback of these methods is that the processing of the residual biomass for further valorization becomes difficult due to various factors, such as chemical and thermal alteration of the target product or emulsification of all component fractions. Some of these disruption methods cannot be scaled up properly to process large amounts of microalgae because they create cell debris that is hard to separate in industrial settings and they can also generate a fair amount of heat that might be detrimental to the compound of interest Carullo et al. (2018), Kapoore et al. (2018). Among the abovementioned methods, HPH and HSH are well-established techniques for algae biomass processing, which have been proven for processing of yeast and bacteria suspensions. Nevertheless, a comparison of the energy consumption by different disruption techniques is a difficult task since each application has specific and individual evaluations.

The specific energetic consumption for pre-treatment of microalgae biomass is crucial for the final process economics and sustainability. In the case of bead milling, the specific energy consumption for disruption of *C. vulgaris* with a cell disintegration degree higher than 80% is in the range of 7.5–10.0 $kWh \cdot kg_{DW}^{-1}$ (DW, dry weight), as reported by Doucha and Livansky (2008). Postma (2017) reported that a selective protein extraction can reduce energy consumption while maintaining the protein yield over 30% (related to dry biomass). In this case, only water-soluble proteins were obtained. In this case, the specific energy consumption was higher than 0.8 $kWh \cdot kg_{DW}^{-1}$. It is also difficult to estimate the energy consumption of HPH, because it depends on various factors such as algal species, the type of the equipment, required degree of disintegration, biomass concentration, etc. According to Samarasinghe et al. (2012), the energy requirement for processing 1 m^3 of algal slurry (100 $g_{DW} \cdot kg_{sus}^{-1}$) in a single pass at low pressure (690 bar) is 69 MJ, which corresponds to specific energy consumption of 0.2 $kWh \cdot kg_{DW}^{-1}$. This treatment resulted in a degree of disintegration lower than 12%, for *Nannochloris oculata*. In order to achieve a higher degree of disintegration (>90%), at least three passes and higher pressures (>2000 bar) are required, which results in a specific energy consumption of 1.7 $kWh \cdot kg_{DW}^{-1}$. In our lab, we used the Avestin EmulsiFlex-C3 HPH with a pressure of 2 kbar as a benchmark procedure for cell disintegration. The specific energy consumption of this HPH can be calculated by taking into account the electric power (0.75 kW) at 2 kbar pressure. Accordingly, the specific energy

demand for a single pass at a flow rate of 0.83 $ml \cdot s^{-1}$ and a biomass concentration of 100 $g_{DW} \cdot kg_{SUS}^{-1}$ is 2.5 $kWh \cdot kg_{DW}^{-1}$. On industrial scale at higher throughput (1000 $l \cdot h^{-1}$), the energy requirement drops significantly when compared with laboratory equipment, such as in the case of the HPH equipment from GEA Westfalia (see Table 1), with a specific energy consumption in the range of 1.1–2.2 $kWh \cdot kg_{DW}^{-1}$.

In contrast to the disintegration methods mentioned before, the PEF treatment is not breaking the cell envelope, and therefore it is considered as a mild cell disruption method. Nevertheless, recent publications have shown the strong potential of PEF as a pre-treatment method to extract intracellular components, such as lipids, proteins, and pigments from microalgae (Goettel et al. (2013), Eing et al. (2013), Luengo et al. (2014), Pataro et al. (2017), Silve (2018a, b), Jaeschke et al. (2019), Scherer et al. (2019), Akaberi et al. (2020)). In these studies, the algae suspension was treated with a specific energy in the range of 50–150 $kJ \cdot kg_{SUS}^{-1}$. As a result, for a biomass concentration of the algae slurry between 10–100 $g_{DW} \cdot kg_{SUS}^{-1}$, the specific energy consumption for PEF treatment is in the range of 0.14–4.2 $kWh \cdot kg_{DW}^{-1}$. Table 1 gives an overview of the specific energy consumption for pre-treatment of microalgae, especially *C. vulgaris*, using various HPH equipment, bead milling, and PEF treatment. In view of these findings, the specific energy consumption does not seem to be decisive reason for the choice of the disintegration method. Furthermore,

Table 1 Specific energy demand of various cell disintegration methods using different equipment for microalgae biomass

Method/ biomass concentration in $g_{DW} \cdot kg_{SUS}^{-1}$	Equipment	Algae species	Degree of cell disintegration in %	Specific energy consumption in $kWh \cdot kg_{DW}^{-1}$	References
HPH 100	*NanoDeBEE* 0.69 kbar, 3 passes	*N. oculata*	> 90	0.6–1.7	Samarasinghe et al. (2012)
	Avestin EmulsiFlex –C3 2 kbar, 5 passes	*C. vulgaris*	–	12.5	–
	GEA Ariete NS3015H 0.4–1.0 kbar, 3 passes	*C. vulgaris*	–	0.3–0.4	SABANA e-bulletin No. 4, 2019
Bead milling dry biomass	Dyno-Mill	*C. vulgaris*	>80 –	7.5–10.0 0.8	Postma (2017)
PEF 5	Transmission line pulse generator: 40 $kV \cdot cm^{-1}$, 1 µs, square pulses	*A. protothecoides*	–	0.3–0.8	Goettel et al. (2013)

it gives priority to select the method, which optimizes the utilization of the entire algal biomass by integration in a biorefinery concept.

Biorefinery Concept Based on PEF Technology

PEF technology opens the possibility for a cascade biorefinery in which all fractions can be successfully recovered, as reported in several studies (Wijffels et al. (2010), Kotnik et al. (2015), Goettel et al. (2013), Eing et al. (2013)). According to the biorefinery concept, the key feature of an alternative pre-treatment method should be the ability to extract high valuable intracellular components from a wet biomass prior to the recovery of lipids by organic solvents. Thus, the degradation of intracellular components is avoided, and the extremely costly drying step is removed from the production line (Xu et al. (2011), Lardon et al. (2009)). Figure 1 shows the microalgae valorization concept based on PEF technology, in which the cell permeabilization is followed by two stages of biorefinery. During the first stage, high-value water-soluble compounds, such as proteins, polysaccharides, and pigments, are recovered, while in the second stage, the lipids are extracted via a green organic solvent (e.g., ethanol). Finally, the residual biomass can be used in energetic processes (e.g., thermochemical conversion (Guo et al. (2019))) or further valorized (e.g., anaerobic digestion).

Although the use of PEF technology for lipid extraction has been demonstrated in various studies (Eing et al. (2013), Goettel et al. (2013), Silve (2018a, b)), an efficient extraction of proteins from microalgae using PEF treatment has not been shown. In fact, some studies have claimed that protein extraction yield is usually too low for PEF treatment to be a feasible option for industrial-scale applications (Postma (2016), Safi (2017), Zocher (2016)). Therefore, in the last years, many efforts have been done to identify the most economical pathway – e.g., suitable PFE treatment and extraction methods for protein recovery.

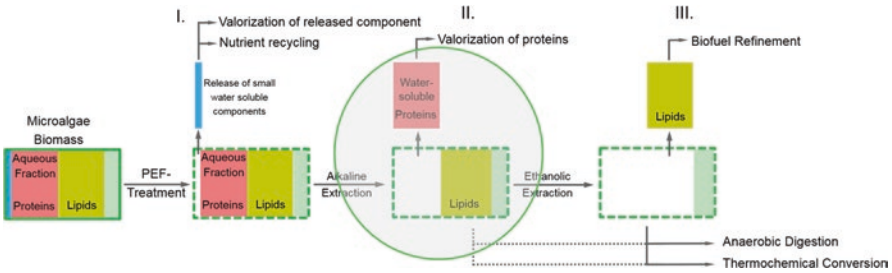

Fig. 1 Biorefinery concept based on PEF technology in which all biomass fractions are used efficiently

Impact of Various Processing Conditions on Recovery of Proteins and Pigments

The main advantage of PEF-assisted extraction is the possibility to get higher degree of extraction selectivity toward certain valuable fractions such as proteins and lipids. In general, PEF treatment of pre-concentrated biomass has to be performed with pulses of a duration of less than 10 μs. Longer pulses cause biomass deposition at the electrodes and may result in clogging of treatment chambers (Sträßner et al. (2016)). In the case of lipid extraction for biofuel production, PEF treatment has shown promising results for reducing the environmental and economic costs of the process (Silve et al. (2018a)). For example, up to 92% of the total lipid content was recovered after PEF treatment and ethanol/hexane extraction of mixotrophically grown *Auxenochlorella protothecoides*. Moreover, it was shown that the lipid yield obtained after PEF treatment with low treatment energy of 0.07 $kWh \cdot kg_{DW}^{-1}$ and 20 h of incubation period was the same as the lipid yield as right after the treatment with high specific energy of 0.42 $kWh \cdot kg_{DW}^{-1}$ without any incubation time (Silve et al. (2018b)). Even though PEF treatment did not cause a spontaneous release of lipids, the efficiency of lipid recovery is much higher than that of proteins, probably due to the combination of the extraction with organic solvents that can penetrate the cells and dissolve the lipids. In contrast to lipids, which are stored intracellularly mainly in oil droplets, proteins are located either in cytoplasm or linked to membranes. Therefore, some authors (Coustets et al. (2014)) proposed that PEF treatment only leads to the release of water-soluble cytosolic proteins without affecting vacuole membrane integrity. They also found that an incubation step in a salty buffer is necessary for an effective recovery of proteins, but they have not identified the parameters, which might influence the protein release. Therefore, the following section pays particular attention to the impact of various treatment parameters on the efficiency of protein recovery from *C. vulgaris* and *A. platensis*, as examples.

Both microorganisms are certified for food and feed application as they are generally regarded as safe (GRAS status) and are being marketed as food additives since many years. The idea to use microalgae as dietary supplement is not new (Morimura and Tamiya (1953)) and is owing to the high protein content of 42–58$\%_{BDW}$ in *C. vulgaris* and up to 70$\%_{BDW}$ in *A. platensis* (Morris et al. (2008), Seyfabadi et al. (2011), Servaites et al. (2012), Safi et al. (2013)). Among various proteins, phycobiliproteins like C-phycocyanins are supplementary light-absorbing complexes (pigments) that are present in high concentration in *A. platensis* (up to 20$\%_{BDW}$) (Safaei et al. (2019)). In addition, the amino acid profile of these proteins has been shown to be similar to that found for egg proteins (Safi et al. (2014)). Dietary supplementation with *C. vulgaris* and *A. platensis* is considered to have positive benefits for human health (Soheili and Khosravi-Darani (2012), Panahi et al. (2015)). For instance, *C. vulgaris* can help in lowering serum cholesterol (Ryu et al. (2014)) and thus is considered to have preventive effects in cardiovascular diseases. Some authors supposed that consumption of *C. vulgaris* has a preventive effect on diabetes as it lowers blood sugar level and mitigates insulin resistance

(Cherng and Shih (2005), Lee and Kim (2009)). Phycocyanins from *A. platensis* are mostly used as color supplements in food and cosmetics (Fernández-Rojas et al. (2014)) and have shown to exhibit antioxidant activity, being associated to the decrease of the risk of degenerative, neuro-, and renal diseases (Li et al. (2015), Memije-Lazaro et al. (2018), Park et al. (2015), Raja et al. (2015)). In the following, the impact of incubation time after PEF treatment, temperature, pH value, and biomass concentration on phenomena that underlie the process of protein release is discussed.

Impact of Incubation Time and Temperature

PEF treatment enables protein release via diffusion, which is strongly time and temperature dependent. Up to 50% of the protein content from *C. vulgaris* can be recovered after PEF treatment with specific energy of 150 $kJ \cdot kg_{SUS}^{-1}$ (40 $kVcm^{-1}$, 1 µs square pulses, 5–10 $g_{DW} \cdot kg_{SUS}^{-1}$) and 24 h incubation period as reported by Scherer et al. (2019). The incubation temperature had an unexpected impact on the release kinetics of proteins from *C. vulgaris* (Fig. 2). According to this study, the optimal temperature for protein release was in the physiological temperature range between 20 °C and 40 °C. The incubation at higher temperatures, above 40 °C, does not lead to faster release, as would be expected for a diffusion-driven process. At 50 °C, the release again was limited, and the yield over the 24 h incubation period was only half of what was obtained under physiological conditions. The authors of

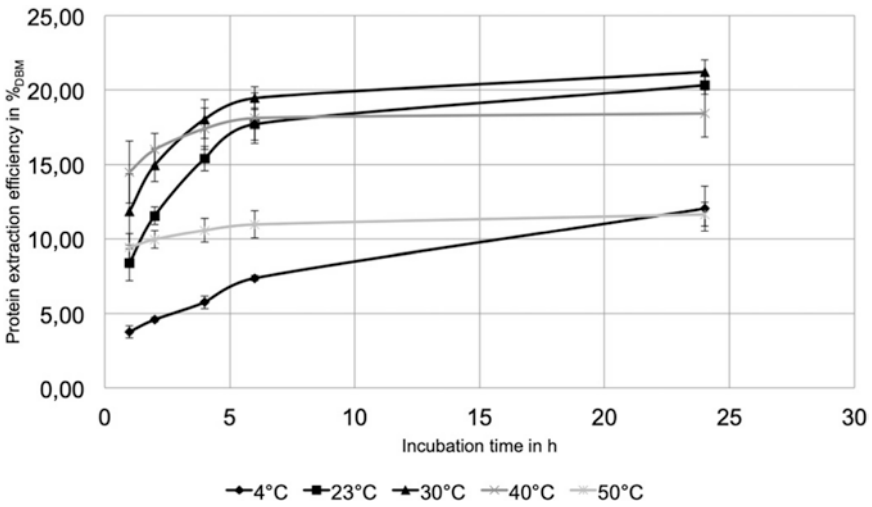

Fig. 2 Time course of protein recovery efficiency after PEF treatment of *C. vulgaris* in dependence of incubation temperature. The microalgae suspension (5 $g_{DW} \cdot kg_{SUS}^{-1}$) was treated with a specific treatment energy of 150 $kJ \cdot kg_{SUS}^{-1}$ and then incubated at different temperatures (Figure: Scherer et al. (2019))

this study considered that besides diffusion, the protein release is enzyme-mediated. In this model enzyme-driven protein release, PEF treatment induces irreversible membrane electroporation in *C. vulgaris* and consequently cell death, followed by self-digestion (autolysis), which results in protein liberations. Similar mechanisms, in which the release of proteins and pigments is facilitated through an autolytic process associated with programed cell death, have been proposed and described for yeast (Simonis et al. (2017), Martinez et al. (2018a)) and red algae *Rhodotorula glutinis* (Martinez et al. (2018b)). The overall results suggest that proteins are released by a proteolytic activity after triggering cell death via PEF treatment. These outcomes are supported by the observed DNA laddering, which is one of the indications for programmed cell death. In *C. vulgaris,* DNA laddering begins within 1 h after PEF treatment and progresses over time, with the genomic DNA completely fragmenting within 24 h, as reported by Scherer et al. (2019). This is in line with protein release, which also begins within 1 h after PEF treatment. In addition, Western blot analysis of water-soluble protein fractions obtained after PEF treatment reveals that proteins from all the organelles are released in the supernatant following treatment. A signal for RuBisCo, histone H3, and actin can be detected within 1 h after PEF treatment (Scherer et al. (2019)), while the signal for COXII, a protein present in mitochondrion, appears after an extended period of 6 h post-PEF treatment. These results show that all intracellular compartments are digested and proteins and pigments can be released from the entire cell.

For *A. platensis* the kinetics of released proteins and C-phycocyanin into the external medium was also strongly influenced by incubation temperature after PEF treatment with 56–114 $kJ \cdot kg_{SUS}^{-1}$ (40 $kV \cdot cm^{-1}$, 1 µs square pulses, 2.6 $g_{DW} \cdot kg_{SUS}^{-1}$), as reported by Akaberi et al. (2020). In contrast to *C. vulgaris*, the release of intracellular components from *A. platensis* is dominated by diffusion. In the first hour after treatment, over 70% of the total protein content was recovered. After 4 h of incubation at either 23 or 40 °C, the maximum protein yield (~97%) was reached. For these temperatures, at least 2 h of incubation were required to obtain the comparable amount of proteins as obtained via HPH treatment (60%$_{BDW}$). The biomass incubated at 4 °C showed a drastically lower protein release, which cannot be compensated even after a longer incubation period of 24 h. This might be an indication of inhibited autolysis in *A. platensis* after cell death.

C-phycocyanin release after PEF treatment was also strongly time and temperature dependent. The amount of C-phycocyanin released after 1 h of incubation at 23 and 40 °C (5.5%$_{BDW}$ and 4.9%$_{BDW}$, respectively) was comparable. After 4 h of incubation, C-phycocyanin concentration reached the same level as those obtained after HPH treatment (up to 10%$_{BDW}$). A drawback of a long incubation time is that at high temperatures (> 40 °C) and unfavorable pH changes in the incubation medium, molecular degradation of C-phycocyanin can happen.

In short, post-PEF incubation temperature strongly influences the protein release from *C. vulgaris* as well as *A. platensis*. A recommended incubation temperature for both microorganisms is in the physiological range between 23 and 30 °C, which can be applied to other microorganisms in a first approach. Significant protein recovery can be achieved for both microorganisms by an incubation period of at least 5 h for

C. vulgaris (50% of total proteins) and 2 h for *A. platensis* (98% of total proteins) after PEF treatment.

Impact of Post-PEF Incubation pH

The protein conformation changes between different thermodynamic states when the incubation conditions (temperature, pH) are altered. By changing the pH of the medium, the hydrogen bonds and the salt bridges dissolve, which change the folding and the solubility of proteins. Accordingly, the pH of the incubation medium and the stability of pH over time are important factors, which can affect the release and conformation of proteins during the incubation period.

Protein recovery efficiency from *C. vulgaris* at various pH values shows that the extraction yield in incubation medium is low at pH 7, but it has an extraction optimum at pH 8–9 and it decreases slightly with further increasing of the pH (Fig. 3). The initial pH of the medium changes during longer incubation, due to the permanent release of cytosolic components and to the ongoing autolytic process. Therefore, for longer incubation times, the pH of the medium must be stabilized using a buffer system, such as Tris.

The efficiency of protein recovery from *A. platensis* suspension (3.6 $g_{DW} \cdot kg_{SUS}^{-1}$) after PEF treatment (40 $kV \cdot cm^{-1}$, 1 µs square pulses, 56–114 $kJ \cdot kg_{SUS}^{-1}$) was also pH dependent, as reported by Akaberi et al. (2020). Incubation in pH 6 and pH 8

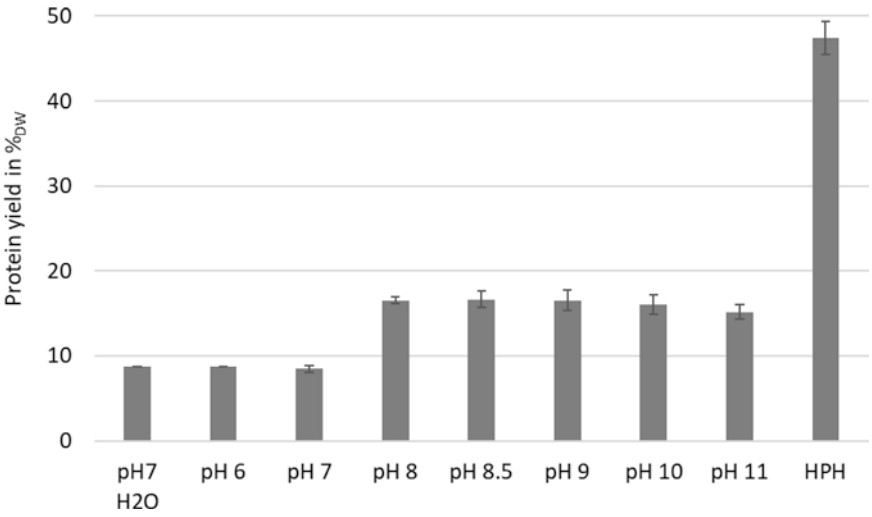

Fig. 3 Protein recovery efficiency from *C. vulgaris* at various pH values. The algae suspension (5 $g_{DW} \cdot kg_{SUS}^{-1}$) was PEF treated (40 $kV \cdot cm^{-1}$, 1 µs, spec. energy of 150 $kJ \cdot kg_{SUS}^{-1}$ means 8.4 $kWh \cdot kg_{DW}^{-1}$) and afterward incubated for 24 h at room temperature under the influence of different pH values in water or leftover medium conditioned to defined pH values

buffer showed a gradual increase of released proteins within 24 h, with 40.5%$_{BDW}$ and 47.6%$_{BDW}$, respectively. In comparison, the total protein content, obtained by HPH treatment of *A. platensis* suspension, was 56.7%$_{BDW}$. When using initial buffer as an incubation medium, the kinetics of protein release was fast at the beginning and decreased gradually after a certain time. At low biomass concentration (3.6 $g_{DW} \cdot kg_{SUS}^{-1}$), a drastic shift in pH from 10.5 to 7.0 within 24 h was observed during incubation in initial medium. This was due to the low buffering capacity of the initial buffer and the tremendous release of intracellular compounds into the medium. Regarding C-phycocyanin recovery, incubation in a buffered medium with a pH of 6 or 8 prevents the molecular degradation of protein complex. At pH 8, more than 97% C-phycocyanin (10.5%$_{BDW}$) was recovered within 3 h after PEF treatment (Akaberi et al. (2020)). The blue color of C-phycocyanin fades drastically when the *A. platensis* suspension was incubated in initial buffer without buffering capacity.

In summary, the monitoring and adjustment of the pH are required in the downstream processes, especially when an incubation step is carried out, in order to maintain the quality of the extracted component and to ensure efficiency of extraction. For *C. vulgaris* suspensions, the protein release is effective at alkaline pH of around 8.5–9 in a buffered environment, whereas a constant pH of 8 is required for *A. platensis*, when high biomass concentrations are used.

Impact of Biomass Concentration

Similarly to HPH, concentrating the biomass before the treatment enables to reduce considerably the energy input per kg of dry biomass. However, the biomass concentration of the microalgae slurry can affect both the PEF treatment and the diffusion during post-PEF incubation period. Due to the high biomass concentration before PEF treatment, cells can aggregate into cluster, which leads to electrical shielding of the cells inside the cluster and thus to a lower membrane electroporation. In addition, the high amounts of externalized components lead to molecular degradation due to redox reactions or enzymatic digestion, which can affect the targeted product.

Experiments with *C. vulgaris* suspensions of different biomass concentrations (36–167 $g_{DW} \cdot kg_{SUS}^{-1}$) revealed that the suspension's biomass content had no negative influence on the efficiency of the PEF treatment as reported by Goettel et al. (2013). It seems that at these biomass concentrations and an electric field intensity of 40 $kV \cdot cm^{-1}$, the so-called percolation threshold is not exceeded (El Zakhem et al. (2006)), and therefore mutual electric shielding is negligible. However, this is not the case when dense *A. platensis* suspension is PEF treated. The kinetics of protein/C-phycocyanin release is considerably influenced by the homogeneity of the suspension (Akaberi et al. (2020)). The long filamentous structure of *A. platensis* is prone to clump together and form large aggregates of cells. Homogeneous suspensions have a faster release kinetics when compared with heterogeneous suspensions. The slow release of proteins and C-phycocyanin in the case of a heterogeneous suspension (containing clusters of cells) is attributed to the mutual electric

shielding of cells, which causes a decrease in the amplitude of induced transmembrane voltage (Guittet et al. (2017)). Consequently, higher specific treatment energies are required for these suspensions, in order to maintain the extraction efficiency. For these reasons, a gentle homogenization of cell suspension prior to the PEF application is recommended in order to avoid cluster formation.

Besides the mutual shielding effect at high biomass concentrations, the release of components and the quality of the extract are also affected. For instance, with increasing the biomass concentration of *C. vulgaris* suspension from 2.5 to 12.5 $g_{DW} \cdot kg_{SUS}^{-1}$, the protein yield after an incubation step of 24 h decreased significantly (from 50% to 30%) (Fig. 4). This can be explained by the fact that biomass concentration influences the diffusion gradient of the intracellular proteins into the medium. With regard to total protein release and C-phycocyanin release from *A. platensis* at higher biomass concentrations (~ 10 $g_{DW} \cdot kg_{SUS}^{-1}$), the following can be stated: In both cases at high biomass concentration, the release kinetics is slower, and the degradation of C-phycocyanin in the medium no longer occurs. Another observation is that the maximum protein and C-phycocyanin yield can only be obtained after an incubation time of 5 h in minimum. In contrast, comparable yields can be achieved after 2 h of incubation when low biomass concentrations were processed. This prolonged incubation time at higher biomass concentrations can be explained by a lower contribution of diffusion to component externalization at higher cell density.

The impairment of the component release and quality with increased cell density represent a new challenge for upscaling in industrial applications and must be clarified in order to maintain the extraction efficiency.

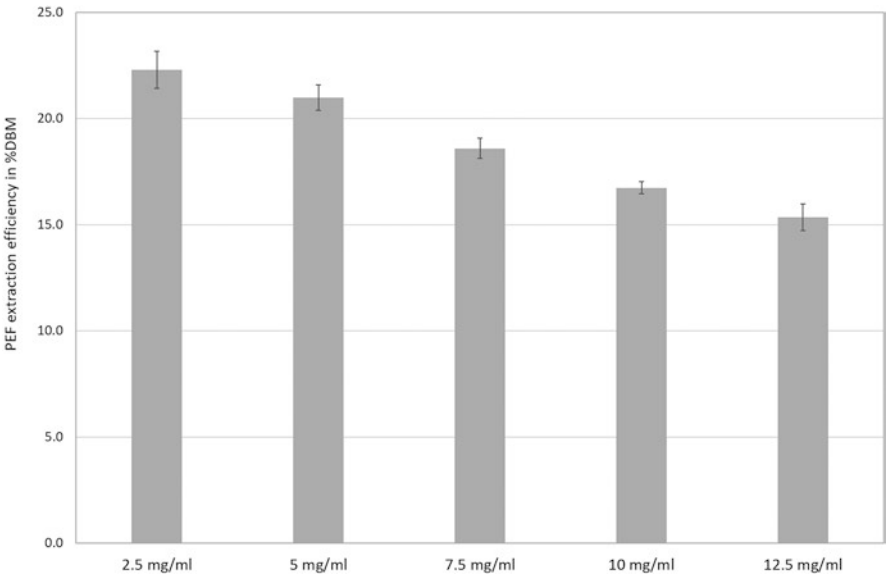

Fig. 4 Protein recovery efficiency from *C. vulgaris* in dependence of biomass concentration. *C. vulgaris* suspensions were PEF treated (40 $kV \cdot cm^{-1}$, 1 µs square pulses, 150 $kJ \cdot kg_{SUS}^{-1}$) and incubated for 24 h at room temperature (Figure: Scherer et al. (2019))

Specific Treatment Energy

The specific treatment energy is a decisive factor for the successful implementation of PEF technology in the large-scale processing of microalgae. The energy required for efficient PEF treatment of microalgae is directly related to the volume of suspension and its properties such as conductivity and biomass concentration but also to the microalgae species and the target product.

As discussed in the previous section, the reduction in energy requirements can be achieved by an additional incubation step, which fosters the release of valuable components after PEF treatment. In this approach, PEF treatment induces cell death, which triggers an autolytic process. Accordingly, the lowest energy demand required for inducing autolytic processes in *C. vulgaris* equals to the specific lethal energy dose. Under this conditions the required PEF treatment energy could be insignificantly low, since the lethal dose for *C. vulgaris* is in the range of 1.4 $kJ \cdot kg^{-1}$ (for 1 µs square pulses of 40 $kV \cdot cm^{-1}$) (Gusbeth et al. (2013)), which corresponds to an energy consumption of only 0.004 $kWh \cdot kg_{DW}^{-1}$, for an algae suspension of 100 $g_{DW} \cdot kg_{SUS}^{-1}$.

Since in the case of *A. platensis*, the autolytic process after PEF treatment can be neglected and diffusion predominates, the lowest treatment energy required for an efficient protein release does not depend on the lethal dose. The kinetic of the protein/C-phycocyanin release is not only time and temperature dependent but also dose dependent. With a high specific treatment energy 114 $kJ \cdot kg_{sus}^{-1}$ (corresponds to 3.2 $kWh \cdot kg_{DW}^{-1}$, for 10 $g_{DW} \cdot kg_{SUS}^{-1}$), the maximum protein yield is reached within 3–5 h, while with 56 $kJ \cdot kg_{sus}^{-1}$ it takes more than 20 h.

In summary, these two examples clearly show that the energy demand of efficient PEF pre-treatment to obtain valuable intracellular components at high quality and in large quantities must be identified for each microorganism and individual purposes.

Conclusion

This approach uses an incubation step after PEF treatment to increase the efficiency of extracting valuable intracellular components. As example, the time-dependent release of proteins and pigments is affected by process factors such as biomass concentration, post-PEF incubation temperature, and pH. Using *A. platensis*, the most important benefit that PEF treatment offered by subsequent incubation in pH 8 buffer is the enhancement of the purity ratio of C-phycocyanin. For some algae species, such as *C. vulgaris*, the induced cell death is sufficient to trigger an autolytic process, which boosts the protein release. By incubating the microalgae suspension after PEF treatment under appropriate conditions (temperature between 20 °C and 40 °C and pH in the range from 8.5 to 9.5), up to 50% of the total protein content can be obtained. This allows a significant reduction of energy consumption and hence reduction of operation costs. From a bioprocess engineering point of view,

this approach opens the opportunity to use PEF treatment in a biorefinery concept, where water-soluble ingredients are extracted before solvent extraction of lipids.

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