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Effect of pulsed electric fields and high pressure homogenization on the aqueous extraction of intracellular compounds from the microalgae *Chlorella vulgaris*

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ABSTRACT

Pulsed Electric Fields (PEF) and High Pressure Homogenization (HPH) are promising and scalable cell disruption technologies of microalgae cells. In this work, the permeabilization degree, morphological properties, and extractability of intracellular compounds from microalgae *Chlorella vulgaris* suspensions (1.2%, w/w) were investigated as a function of PEF treatment at different electric field strengths (10–30 kV/cm) and total specific energy input (20–100 kJ/kg), in comparison with the more disruptive HPH treatment (150 MPa) at different number of passes (n_P = 1–10). The conductivity and the particle size analyses, as well as the SEM images, clearly showed that PEF induces the permeabilization of the cell membranes in an intensity-dependent manner, without producing any cell debris, whereas HPH treatment causes the total disruption of the algae cells into small fragments. Coherently with the lower permeabilization capability, PEF promoted the selective extraction of carbohydrates (36%, w/w, of total carbohydrates), and low molecular weight proteins (5.2%, w/w, of total proteins). On the other hand, HPH induced the undifferentiated release of all the intracellular content, resulting in a 1.1 and 10.3 fold higher yields than PEF, respectively of carbohydrates and protein.

These results suggest that, in a multi-stage biorefinery, PEF could represent a suitable cell disruption method for the selective recovery of small-sized cytoplasmic compounds, while HPH should be placed at the end the cascade of operations allowing the recovery of high molecular weight intracellular components.

1. Introduction

Chlorella vulgaris is a freshwater eukaryotic microalga with a mean diameter ranging from 2.5 to 5 μm [1] belonging to the division of Chlorophyta. It has drawn large attention over the last decades because of its capability to accumulate large amounts of valuable components, especially proteins (51–58%), but also polyunsaturated fatty acids (14–22%), carbohydrates (12–17%), nucleic acids (4–5%), vitamins and minerals [2,3]. Moreover, it accumulates also chlorophyll (1–2%) that imparts the characteristic green color, masking the other less concentrated pigments, such as lutein and other carotenoids [4]. The extraction of all these intracellular compounds, which can be used as natural additives or active ingredients for food, cosmetic, pharmaceutical and animal feed products, as well as in the production of biofuels [5,6], is crucial for achieving an economically feasible microalgae biorefinery [7].

However, these compounds are located in different parts of the cells, protected by the rigid cell wall and membranes surrounding the cytoplasm and the internal organelles (e.g., chloroplast), which greatly limit their rate of mass transfer during extraction. Conventional extraction processes of these intracellular compounds are often conducted from dry biomass with organic or aqueous solvents, depending on the polarity of the target compounds [8,9]. However, these methods suffer from several limitations, namely the long extraction times and the use of relatively large amounts of solvent, and may lead to the co-extraction of undesirable components, with increased downstream processing costs [7,10]. In addition, the drying of microalgal biomass is reported to be one of the major energy-consuming steps within the overall process and is responsible for significant losses of valuable compounds [5,7].

For these reasons, the application of conventional or innovative cell disruption methods to wet biomass may considerably promote the implementation of the biorefinery concept on microalgae, enabling a

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faster and more efficient release of intracellular compounds at low temperature. This also contributes to limit the degradation of the extracts and promotes the reduction of energy costs, of solvent consumption, as well as of the extraction time [7,10].

Among the cell disruption methods, the Pulsed Electric Fields (PEF) and the High Pressure Homogenization (HPH) treatments have emerged as promising methods for the mild and complete disruption of biological cells, respectively [9–14]. Moreover, both PEF and HPH can be easily scaled up to process large volumes of wet biomass in a wide range of solids concentration, thus avoiding the need for energy-intensive drying and possibly allowing to reduce the energy demand per unit biomass [5,15–18].

In PEF processing, the biomaterial is placed between two electrodes of a treatment chamber and exposed to high intensity electric fields (10–50 kV/cm), applied in the form of repetitive pulses of very short duration (from several nanoseconds to few milliseconds), which induce the permeabilization of cell membranes by electroporation, facilitating the subsequent release of intracellular matter [19]. Several studies highlighted the effectiveness of PEF to enhance the selective recovery of intracellular compounds from wet microalgal biomass, including lipids [20,21], pigments [8,10,14,22–23], carbohydrates, and water-soluble proteins of small molecular weight [6,9,14,18,23].

However, the extraction of molecules of higher molecular weight, or more bounded to the intracellular structure (e.g., proteins), requires the application of more effective cell disruption techniques, such as HPH [10].

HPH is a purely mechanical process, during which a liquid dispersion of plant material or a cell biosuspension is forced by high pressure (50–300 MPa) through a micrometric disruption chamber, where the velocity increases rapidly and the pressure decreases to atmospheric conditions as the suspension exit the unit [15]. As a result, the biological cell suspension is subjected to extremely intense fluid-mechanical stresses (shear, elongation, turbulence, and cavitation), which cause the physical disruption of the cell wall and membranes [16,24,25].

Due to its high cell disruption efficiency [7], HPH is reported to markedly increase the extraction yield of several intracellular compounds from microalgae [7,14,26–28]. However, the HPH treatment causes the non-selective release of intracellular compounds, with the concurrent dispersion of cell debris, complicating the downstream separation processes [14]. Moreover, because of the intense interfacial shear stresses and inherent heating occurring in the homogenization valve, which might induce the degradation of compounds, such as proteins [29–30], HPH treatments always require an efficient heat dissipation at the homogenization valve.

Although several studies have already highlighted the potential of PEF and HPH pre-treatments in the microalgae biorefinery, to date, only the study of Safi et al. [28] has addressed the comparison of their efficiency in terms of cell disintegration, energy input and release of soluble proteins from microalgae *Nannochloropsis gaditana*. However, suspensions of this microalgae were prepared from a frozen paste and at different biomass concentration for PEF (15–60 g_{DW}/L) and HPH (100 g_{DW}/L) treatments.

Moreover, a deeper knowledge regarding the impact of these novel technologies at micro and macro scale is required, which is thoroughly necessary in view of their use in a cascade biorefinery approach of microalgae, where the control of the degree of cell breakage could be exploited to enable the fine tuning of the recovery process of intracellular components [6,7,31].

Therefore, the aim of this study is to investigate comparatively the effects of the main process parameters of both PEF and HPH treatments on the cell disintegration degree, the energy consumption, and the release of intracellular compounds (ionic substances, proteins, and carbohydrates) from fresh *C. vulgaris*, in order to select, for each investigated technology, the best treatment conditions in the perspective of their implementation in a biorefinery scheme.

2. Materials and methods

2.1. Microalgae and cultivation

The microalgal strain used in this study was *Chlorella vulgaris* (CCAP 211), purchased from the Culture Collection of Algae and Protozoa (Argyll, UK). It was cultivated in modified Bold's basal medium [32] at pH 7.0 ± 0.5, in a 5 L horizontal tubular photobioreactor illuminated by four 40 W fluorescent lamps from one side [33]. The composition (per liter of distilled water) of the modified medium was as follows: 1.5 g $NaNO_3$, 0.45 g $MgSO_4{\cdot}7H_2O$, 0.15 g NaCl, 0.45 g $K_2HPO_4{\cdot}3H_2O$, 1.05 g KH_2PO_4, 0.15 g $CaCl_2{\cdot}2H_2O$, 0.003 g vitamin B_1, 7.5 10^{-6} g vitamin B_8, 7.5 10^{-6} g vitamin B_{12} and 6 mL of P-IV solution (Sigma Aldrich, Milan, Italy). The culture was aerated at a rate of 1000 cm^3/min with an air flow containing 2% (*v*/v) carbon dioxide. Growth conditions were monitored by optical density (OD) measurements at 625 nm using a UV–Vis spectrophotometer (Lambda 25 model, Perkin Elmer, Milan, Italy). The pH of the culture medium was monitored during the experiments using a pH meter (pH 211, HANNA Instruments, Woonsocket, RI). Microalgae were harvested during the end of the exponential phase at a biomass concentration of about 3 g_{DW}/L of suspension and then concentrated by centrifugation (centrifuge model 42426, ALC, Milan, Italy) at 4000 ×*g* for 10 min at 20 °C up to a final concentration of 12 g_{DW}/L. The concentrated biomass was pre-packed in high-density polyethylene bottles (Nalgene) cooled at 4 °C, and sent to the laboratories of ProdAl Scarl (University of Salerno, Italy). Samples were transported in an EPS box under refrigerated conditions and delivered within 24 h. PEF and HPH treatments were performed on the delivery day. The initial electrical conductivity of algae suspension was about 1.78 ± 0.03 mS/cm at 25 °C (Conductivity meter HI 9033, Hanna Instrument, Milan, Italy).

2.2. PEF treatment

PEF treatments were conducted in a bench-scale continuous flow PEF unit, described in detail in a previous work [6]. Briefly, the unit consisted of a peristaltic pump to control the flow rate of the algae suspension through the system. The inlet temperature of the algae suspension was controlled using a stainless steel coil immersed in a water heating bath. The PEF treatment zone consisted of two modules, each made of two co-linear cylindrical treatment chambers, hydraulically connected in series, with an inner radius of 1.5 mm and a gap distance of 4 mm. The treatment chambers were connected to the output of a high voltage pulsed power (20 kV–100 A) generator (Diversified Technology Inc., Bedford, WA, USA) able to deliver monopolar square pulses (1–10 μs, 1–1000 Hz). The maximum electric field intensity (E, in kV/cm) and total specific energy input (W_T, in kJ/kg_{susp}) were measured and calculated as reported in Postma et al. [6]. T-thermocouples were used to measure the product temperature at the inlet and outlet of each module of the PEF chamber.

During PEF treatment, the algae suspension (12 g_{DW}/L) was pumped, from a feeding tank under stirring, through the treatment chamber at a constant flow rate of 33.3 mL/min. The pulse length was fixed at 5 μs, while the electric field strength (E) of 10, 20 and 30 kV/cm and total specific energy input (W_T) of 20, 60, and 100 kJ/kg_{susp} were set by varying the applied voltage and the pulse repetition frequency, respectively. All the experiments were carried out at an inlet temperature of each module of PEF chamber of 25 °C, while the maximum temperature increase at the exit of each module due to Joule effect never exceeded 10 °C.

At the exit of the treatment chamber, treated and untreated (without applying PEF treatment) algae suspensions were collected in plastic tubes and placed in an ice water bath to be rapidly cooled up to a final temperature of 25 °C before undergoing the aqueous extraction process.