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Flow Process for Electroextraction of Total Proteins from Microalgae

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Abstract Classical methods for protein extraction from microorganisms, used for large-scale treatments such as mechanical or chemical processes, affect the integrity of extracted cytosolic protein by releasing proteases contained in vacuoles. Our previous experiments on flow-process yeast electroextraction proved that pulsed electric field technology allows us to preserve the integrity of released cytosolic proteins by keeping intact vacuole membranes. Furthermore, large volumes are easily treated by the flow technology. Based on this previous knowledge, we developed a new protocol in order to electroextract total cytoplasmic proteins from microalgae (*Nannochloropsis salina* and *Chlorella vulgaris*). Given that induction of electropermeabilization is under the control of the target cell size, as the mean diameter for *N. salina* is only 2.5 μm, we used repetitive 2-ms-long pulses of alternating polarities with stronger field strengths than previously described for yeasts. The electric treatment was followed by a 24-h incubation period in a salty buffer. The amount of total protein released was evaluated by a classical Bradford assay. A more accurate evaluation of protein release was obtained by SDS-PAGE. Similar results were obtained with *C. vulgaris* under milder electrical conditions, as expected from their larger size. This innovative technology designed in our group should become familiar in the field of microalgae biotechnology.

Keywords Electroextraction · Microalgae · Nannochloropsis · Chlorella · Flow process · Double pulse

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Introduction

Cytoplasmic proteins can be extracted from yeasts by electropulsation [pulsed electric field (PEF) technology] (Ganeva et al. 2001, 2003; Ohshima et al. 1995; Suga et al. 2007; Suga and Hatakeyama 2009; Zakhartsev et al. 2007; Ganeva and Galutzov 1999). Flow-process treatment of yeasts (*Saccharomyces cerevisiae*), with high-intensity electric field pulses, allows the release of the intracellular protein content on large culture volumes (Ganeva et al. 2004). The proof of concept of the flow-process protocol was previously validated (Ganeva et al. 2003) (Fig. 1). Flow-process electroextraction was indeed patented to the CNRS (FR 0013415, Euro/PCT 1982525.6). A preindustrial pilot was developed in our group during the FP7 "Electroextraction" project (FP7-SME-2007-1, grant agreement 222220). Standard methods, such as mechanical disintegration (glass bead grinding) and chemical extraction (lyticase enzyme), classically used for large-scale treatments, affected protein stability (Schuttle and Kula 1990; Naglak et al. 1990). This was due to the disintegration of vacuoles, releasing proteases. This negative step did not occur with PEF, where the specific activity of extracted proteins was always higher than with classical methods (either glass bead disruption or enzymatic treatment) (Ganeva et al. 2003).

Electroextraction is a promising approach for biotechnology. On cyanobacteria (*Synechocystis*), PEF was used to

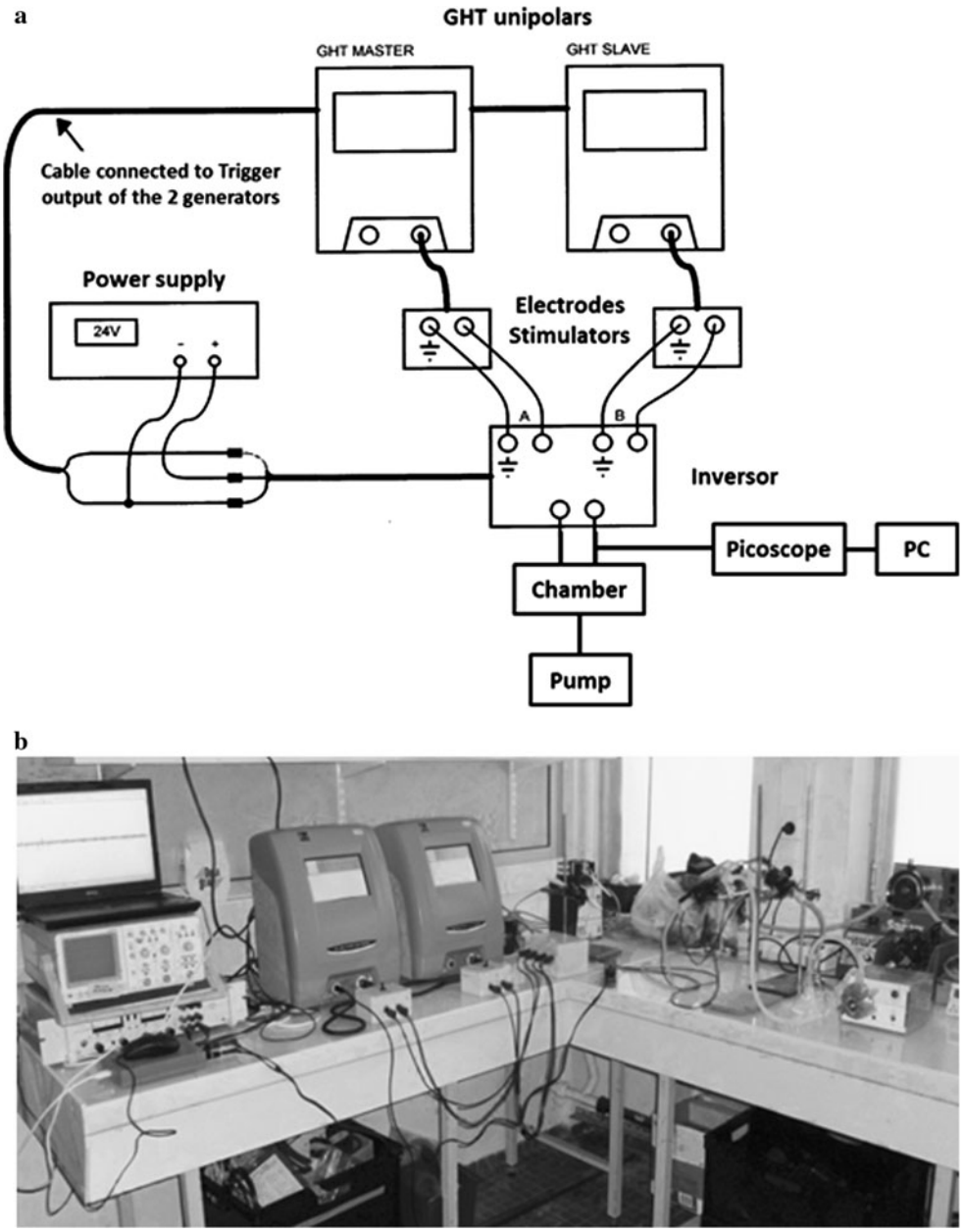


Fig. 1 Scheme and picture of the electroextraction system. Scheme representing the electrical device, where two S20 pulse generators are successively delivering similar pulses with a controlled delay in between to the flow applicator. This is obtained through an inversor that reverses the polarity of the second pulse in order to obtain bipolar symmetric pulses within the flow applicator. The bipolar pulse train is delivered at a frequency higher than 16 Hz

induce cell disruption prior to solvent extraction, using propanol, to extract lipid molecules (Sheng et al. 2011). Another example was given by Kumar et al. (2011), where it was proposed to improve cellulose hydrolysis to sugar from wood chips using PEF. A batch process for RNA extraction was also recently described for the detection of harmful algal blooms (Bahi et al. 2011).

In the present study, using the preindustrial-scale pilot system, a new protocol was checked in order to electroextract total proteins from a large volume of microalgae, taking into account that they were indeed photosynthetic yeasts (Rochaix 1995).

The simple growth requirements of microalgae made these microorganisms attractive bioreactor systems for the production of high-value heterologous proteins. The cultivation could be achieved in photobioreactors which provided accurate control of the culture environment. It was possible to produce recombinant protein in either the cytosol or chloroplast of microalgae (Walker et al. 2005). One of the most recent examples was given by the

production of unique immunotoxin cancer therapeutics in algal chloroplasts (Tran et al. 2013).

Extraction of proteins was diminished by the cell wall barrier. Microalgae with a fragile cell wall did not show significant differences with their protein extract, which was the complete opposite for microalgae having a rigid cell wall; and therefore, disrupting the rigid cell wall of *Chlorella vulgaris* was required to obtain complete protein release after extraction (Safi et al. 2013). Thus, our experiments were performed on microalgae with rigid cell walls.

We further selected a freshwater species, *C. vulgaris*, and a marine one, *Nannochloropsis.* Members of the genus *Nannochloropsis* (Eustigmatophyceae) are pico planktonic algae that were widely distributed. *Nannochloropsis salina* (Fig. 2) is a rather small (diameter 2–5 μm) unicellular marine microalgae, which was investigated for biofuel production. For basic research, the genus *Nannochloropsis* was well suited for a better understanding of pigments' functional organization in higher plants (Mohammady 2011) because, like them, it lacked chlorophyll b and c and contained a high proportion of violaxanthin (carotenoids) (Brown 1987).

Chlorella (Trebouxiophyceae) is a genus of unicellular green microalgae that is ubiquitous in freshwater environments. *C. vulgaris* is bigger than *N. salina* (diameter 3–6 μm) and contains highly nutritious substances such as proteins, vitamins, minerals, fatty acids and nucleic acids (Song et al. 2011).

Field effects on a cell were linked to the size of the target (Sixou and Teissié 1990; Bellard and Teissié 2009a, b). High fields were required to permeabilize small cells and their organelles, such as *N. salina*. Given that the electroextraction system was already optimized for yeasts, the advantage conferred by this new method was to leave vacuole membranes intact (vacuoles contained proteases). With the small-sized *N. salina* (2.5 μm), we were also interested in electroextracting proteins, which were located in the chloroplasts of microalgae (diameter ~1 μm). Due to the size dependence of the PEF effect and the screening effect of the plasma membrane, the smaller organelles required larger field strength than the plasma membrane to induce electropermeabilization (Esser et al. 2010). Previous experiments, at 6 kV/cm, were conducted on bacteria (*Escherichia coli*), which had a mean diameter similar to the chloroplast, to trigger protein electroextraction (unpublished data). Therefore, to permeabilize the saline microalgae, the bacterial parameters would be applied. In light of the results obtained with yeasts, repetitive millisecond-long pulses would be applied to microalgae in order to extract cytosolic proteins. Similar results were obtained with *C. vulgaris* under milder electrical conditions, as expected from their larger size.

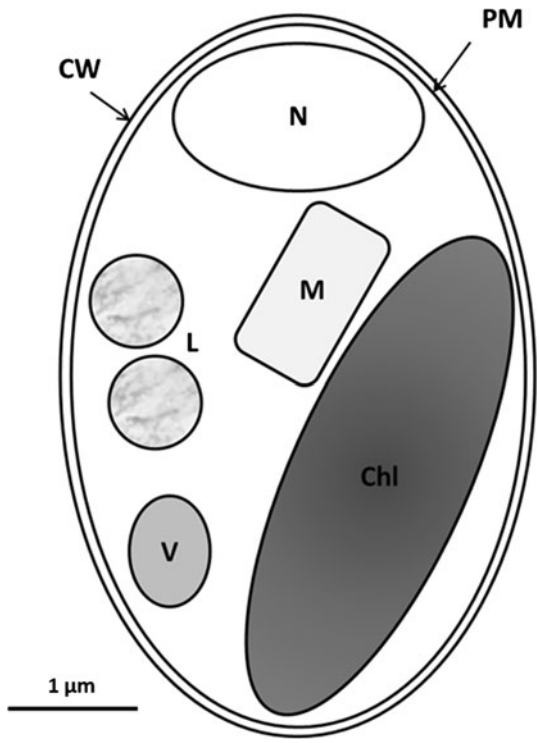


Fig. 2 Schematic representation of *Nannochloropsis*, adapted from Krienitz (2010). *CW* cell wall, *PM* plasma membrane, *N* nucleus, *M* mitochondrion, *Chl* chloroplast, *L* lipid vesicles, *V* vacuole. A well-defined size distribution is present between the different organelles: R MP > R Chl > R V. Electropulsation induced permeabilization of the membrane of closed vesicles in a field- and size-dependent effect. Larger vesicles are permeabilized under milder conditions than for the smaller ones (Sixou and Teissié 1990). Organelles inside the cytoplasm are submitted to a field that is lower than the externally applied one due to the shielding effect of the electropermeabilized plasma membrane (Esser et al. 2010). As a consequence, the plasma membrane is permeabilized when pulsed under field intensities much lower than what is needed for the chloroplasts. The vacuole remains unaffected due to its very small size

The simple electroextraction method allowed a high-yield extraction of total cytosolic proteins from microalgae, as shown on yeasts (Ganeva et al. 2003).

Materials and Methods

Strain and Culture Conditions

The marine microalgae *N. salina* was grown in a closed photobioreactor system (phytobag system; Phytolutions, Bremen, Germany) in an outdoor production site in Bremen, Germany, during the summer months, with mean light conditions of 1,000 mW/m^2. The temperature was controlled by an integrated cooling system to maintain a culture condition of 25 ± 0.5 °C during the day and an adaptation to ambient temperature not lower than

15 ± 0.5 °C during nights. Technical CO_2 was added to maintain a pH value of 7.5 ± 0.3 during cultivation. The medium was a modified *f* medium (Guillard and Ryther 1962) made with artificial seawater at 25 g total suspended solids (TSS)/l salinity. Medium was added to the culture, when required, in order to prevent nutrient limitation. The biomass was harvested at a concentration of 2 g TSS/l with the phytoharvester from Phytolutions to a concentration of 20 g TSS/l.

Chlorella vulgaris cultures were purchased from Teramer (Nimes, France). They were grown in a photoreactor built in the lab (Fig. 3). One large glass bottle was half-filled with sterile water. The bottle was illuminated with a set of three 3-W white LEDs (Bricoman, Toulouse, France) for 12 h a day (timer 190092, Bricoman). The three LED lamps were fixed at three symmetrical positions around the bottle with a tilted orientation (plug 190092, Bricoman) to focus the light beam on the microalgae culture. The temperature was kept around room temperature (~22 °C). A plastic tubing from a CO_2 generator (Biostarter Bio 60; Dennerle, Vinnigen, Germany) was fed to the bottle, to keep CO_2 in the mineral water. Algae fertilizer (KTS001, Teramer) was added to the water, according to the manufacturer's instructions. Stirring was done with a magnetic stirrer (Bioblock, Illkirch, France). The algae starter culture was added and left for 2–3 weeks to allow the algae to multiply. Growth was checked by measuring the density of algae under an inverted microscope with a Malassez slide.

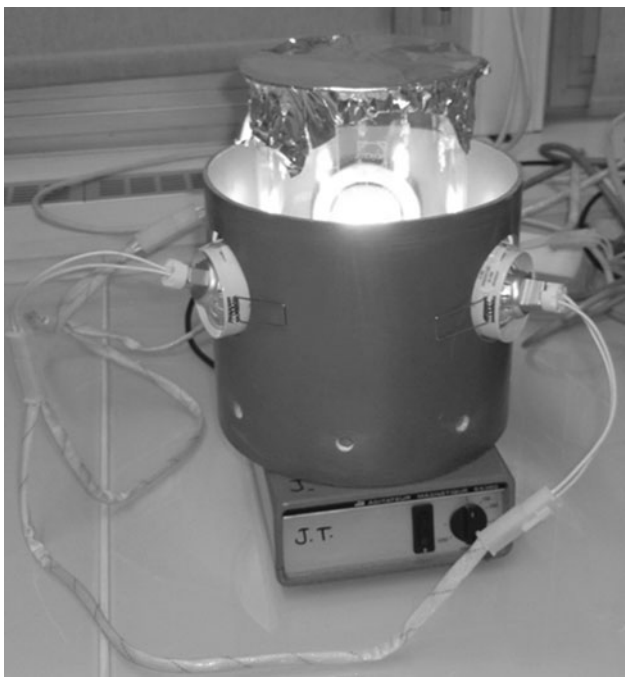


Fig. 3 Photobioreactor. Cells were grown in the bottle sitting in the core of the photosystem with the three LEDs

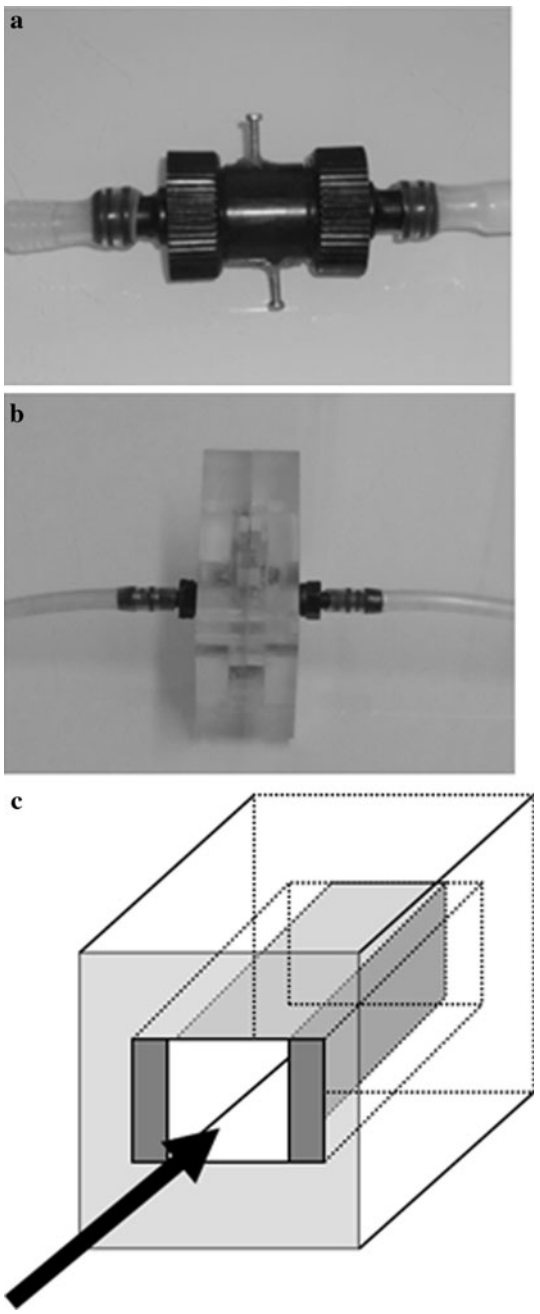


Fig. 4 Flow pulsing chambers. **a** Distance between electrodes $d = 6$ mm, to obtain a field strength of 3 kV/cm. A 4 l/h rate was achievable. **b** Distance between electrodes $d = 3$ mm, to deliver 6 kV/cm. The flow rate was reduced to 0.4 l/h. **c** Geometry of the flow pulsing chamber: two walls of the chamber built in Plexiglas were holding the parallel plate electrodes. The field distribution was focused in the interelectrode space, where it was homogeneous. The electric load was therefore limited to this space

Sample Preparation

A 100-ml volume of *N. salina* suspension in water, at a concentration of 10^8 cells/ml, or *C. vulgaris*, at a concentration of 10^7 cells/ml, was centrifuged at 2,500×*g* for

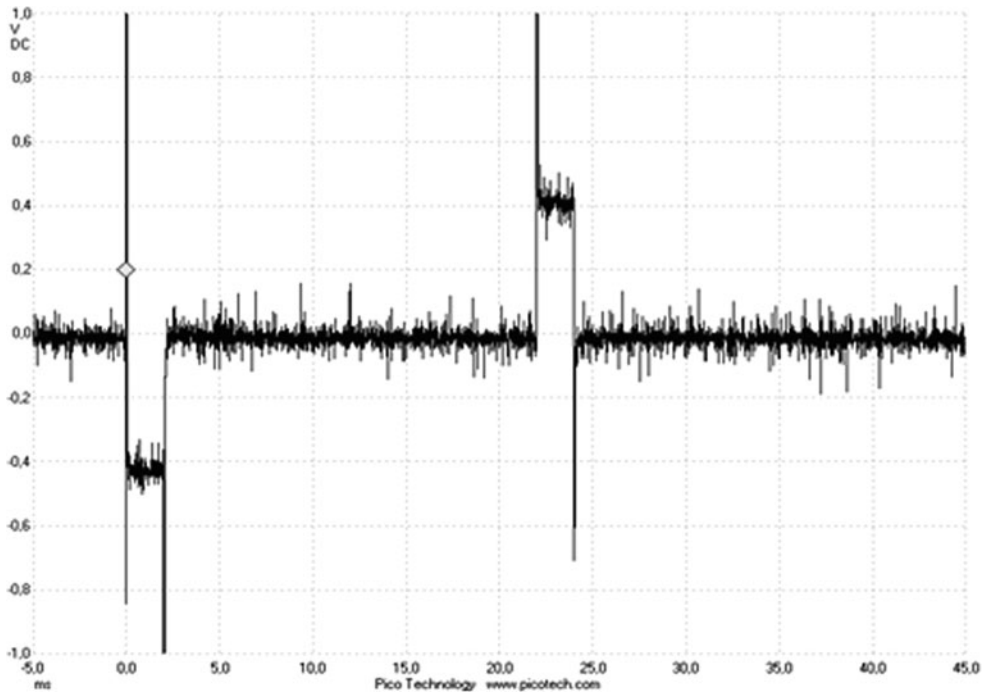


Fig. 5 Current profile. Delivery of the electric pulse train was monitored online with a Chauvin Arnoux current probe on a digitized scope. Symmetrical square waved profiles were observed

5 min at room temperature (~22 °C). Supernatant was removed, and the cells were resuspended in 100 ml of distilled water (final conductivity *N. salina* = 100 µS/cm, *C. vulgaris* = 200 µS/cm) (HI8820N conductimeter; Hanna, Bologna, Italy). This final conductivity was carefully checked before electric treatment.

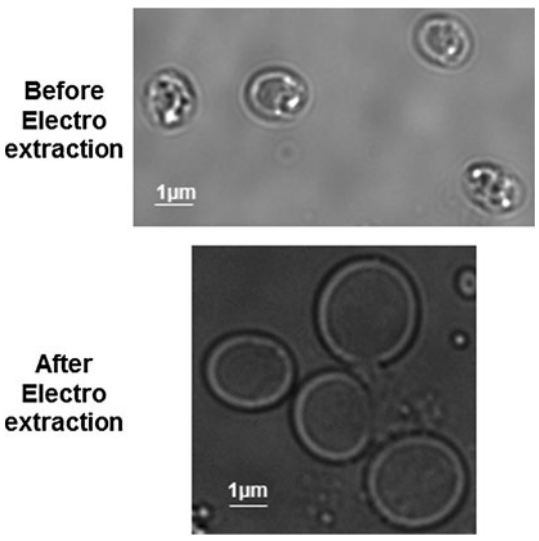


Fig. 6 Phase-contrast microscopic observation of the effect of the electric pulse train. *Nannochloropsis* was observed before and after the electrotreatment

Controls

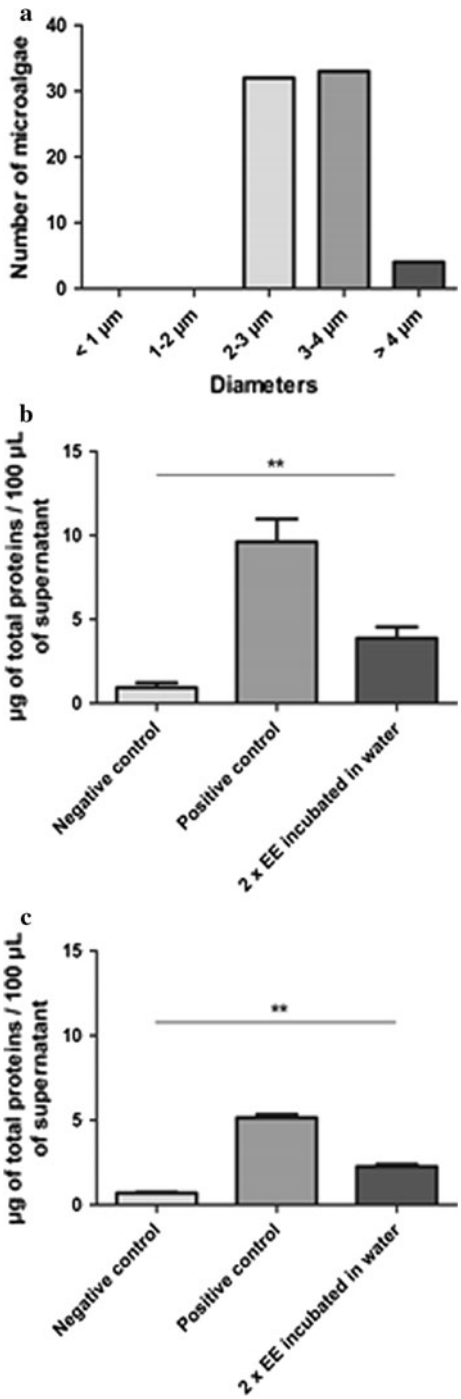
Negative Controls

A sample of 5 ml of *Nannochloropsis* or *Chlorella* washed total suspension was centrifuged at 2,500×*g* for 5 min at room temperature (~22 °C). The pellet was then resuspended in 10 ml of distilled water or phosphate buffer (PB 105 mM, 0.3 M glycerol, 1 mM DTT, pH 7) and incubated at room temperature overnight. The suspension was then centrifuged at 2,500×*g* for 5 min at room temperature. Final analyses were made on the supernatants.

Positive Controls

A sample of 5 ml of *Nannochloropsis* or *Chlorella* washed total suspension was centrifuged at 2,500×*g* for 5 min at room temperature (~22 °C). The pellet was resuspended in 10 ml of methanol (99.8 %) with strong vortex mixing for 15 s. The sample was then incubated at room temperature overnight. The suspension was centrifuged at 2,500×*g* for 5 min at room temperature the next day. Final analyses were made on the supernatants.

Another control was made for migration on SDS-polyacrylamide gel in order to facilitate the loading. *Nannochloropsis* washed total suspension (5 ml) was centrifuged at 2,500×*g* for 5 min at room temperature. The pellet was then resuspended in 5 ml of Dulbecco's PBS (GIBCO-



◀**Fig. 7** Total protein assay for *Nannochloropsis*. **a** Size histogram of the population. **b** The concentration of microalgae suspension in those assays was 1×10^8 cells/ml. Negative and positive controls were made with unpulsed suspensions of microalgae, respectively incubated in water or methanol and vortexed. The remaining suspension in water was processed by a 6-kV/cm electroextraction and then incubated either in water or in methanol. The incubation period of all samples and controls was 24 h at room temperature. Concentrations of total proteins were determined by a classical Bradford assay, using BSA as a standard (see "Materials and Methods" section) ($n = 6$). Statistical analyses were made by Prism software using standard error means and a *t* test. **c** Same as in **b**, but those assays were conducted at a concentration of 5×10^7 cells/ml ($n = 2$). Statistical analyses were made by Prism software using standard error means and a *t* test

Invitrogen, Carlsbad, CA). One milliliter of the suspension was conserved as a negative control. Four milliliters of glass beads (425–600 µm, G8772; Sigma, St. Louis, MO) were added to the other 4 ml of suspension, followed by 1-min vortexing and 30 s on ice, repeated ten times. Then, 8 ml of phosphate buffer (PB 105 mM, 0.3 M glycerol, 1 mM DTT, pH 7) were added to the 2 ml of the recovered extracted suspension and centrifuged at 2,500×*g* for 5 min at room temperature. The supernatant was conserved at 4 °C until final analysis.

Total Protein and Pigment Electroextraction

Pulse generators (Betatech, Saint-Orens-de-Gameville, France) and flow-through applicators (CNRS, Toulouse, France) were designed to apply trains of pulses on the flow. The electric system was made of two S20u generators, each able to deliver up to 2 kV under 6 A (maximum for safety reasons), with adjustable pulse duration of a few milliseconds. Delays between pulses were down to 30 ms. An analog switch was used between the two generators and the pulsing chamber. A pulsing chamber was built of several parts, the most important being the discharge chamber. For *Chlorella* treatment, the distance between electrodes was 6 mm, the height was 6 mm, the length was greater than 1 cm and the volume was up to 1.08 ml (Fig. 4a). It was possible to use several of these discharge chambers to obtain larger discharge chambers or successive separated units (Barbosa-Canovas et al. 1999). The other applicator, used for the *Nannochloropsis* protocol, had a distance between electrodes reduced to 3 mm, a width of 5 mm and a volume of 150 µl (Fig. 4b). This innovative system designed in our group along the FP7 "Electroextraction" project is now commercially available from Betatech. Washed suspensions of *Chlorella* (100 ml) were flow-processed (flow rate = 1 ml/s) through the first pulsing chamber ($d = 6$ mm), according to the parameters determined for yeasts (EEy): $E = 3$ kV/cm, duration of pulses = 2 ms. The number of bipolar impulsions delivered on average to each cell was 15 (i.e., 30 pulses delivered on average per cell during its residency in the pulsing chamber). *Nannochloropsis* treatment was delivered when this suspension was flow-processed (flow rate = 150 µl/s) through the second pulsing chamber ($d = 3$ mm), according to the parameters determined for bacteria (EEb): $E = 6$ kV/cm, duration of pulse = 2 ms. The number of bipolar pulses delivered on average to each cell was 15. After the electric treatment, the pulsed suspension was collected and diluted five times in water or in a phosphate buffer (PB/DTT = PB 105 mM, 0.3 M glycerol, 1 mM DTT, pH 7). Samples were incubated overnight at room

temperature. Supernatants were collected after centrifuging for 5 min at 2,500×*g*.

The electric field was estimated as the voltage applied divided by the distance between the plate electrodes, which was 6 or 3 mm (Fig. 4c).

Total Protein Assays

Protein concentration was determined using a commercial kit (Bio-Rad, Hercules, CA) with bovine serum albumin as a standard (Bradford assay for evaluation of total proteins).

Analytical Gel Electrophoresis

SDS-PAGE of protein samples was performed on a 12 % acrylamide slab gel as described by Laemmli (1970). The sample were preconcentrated by ammonium sulfate precipitation followed by dialysis (dialysis bag cutoff 12000 MVCO, Spectra/Por; Spectrum Laboratories, Rancho Dominguez, CA) against a 25 mM phosphate buffer, pH 7. Silver staining of gels as described by Nesterenko et al. (1994) was made with a protein molecular weight marker (Page-Ruler^{TM} Prestained Protein Ladder; Thermo Scientific, Pittsburgh, PA).

Statistical Analysis

Prism version 5.0 (GraphPad Software, La Jolla, CA) was used to analyze our data. All graphs presented in this article were created on Prism 5.0, using standard error means to obtain the error bars and then *t* tests to compare two sets of data. This statistical method gives an asterisk when the difference between two sets is significant. The asterisk represents the *P* value summary according to Prism.

Results

Microalgae Electropermeabilization

The current delivered by the pulse generator at a given applied voltage was a direct assay of the conductivity of the solution present in the pulsing chamber (Fig. 5). It was observed to be much higher than the value measured before the pulse delivery just after washing the cultures (100 or 200 µS/cm). This high value was further checked by measuring the conductivity of the recovered pulsed samples. This conductivity increase was larger when the microorganism density was brought from 5×10^7 to 10^8 cells/ml. This observation was indicative of the release of the ions present in the cytoplasm as a consequence of the plasma membrane electropermeabilization (Eynard et al. 1992). The same behavior was observed with the two species.

Phase-contrast microscopic observation of the samples before and after electric treatment showed a loss of contrast after pulse delivery (Fig. 6). This was again direct experimental evidence that the cytoplasmic content of the pulsed sample was strongly affected compared to the control.

The pulsation-associated conductivity increase was associated with a higher energy delivery and Joule heating. This could be evaluated by assuming that all the delivered electrical energy was converted in heat in the solution under the stringent assumption that no heat was lost between the pulses during the train. As the pulsed volume was with a simple geometry (flat parallel electrodes), the resistance of the sample was $R = d/\Lambda\ S$, i.e., $0.6/(0.9 \times 10^{-4}) = 3.3 \times 10^3$ ohms, where d is the distance between the electrodes, S their section and Λ the sample conductivity (during the pulse delivery). As 1,800 V was applied, the current was 0.56 A. During the residence of the microalgae, a train of 15 double-polarity, 2-ms-long pulses was delivered. The energy was $1{,}800 \times 0.56 \times (2 \times 10^{-3}) \times (2 \times 15)$ and used to heat a 1-ml volume.

The temperature increase was therefore 15 °C, followed by a fast cooling process as the sample was flowing away from the chamber in the tubes that were at room temperature (22 °C in our lab). This transient temperature jump was limited under the present conditions (5×10^7 cells/ml) and did not affect the protein structure.

Protein Extraction from *N. salina*

A spontaneous protein leakage was obtained from the negative control incubated in water after the overnight incubation. Through the Bradford assay, it was observed

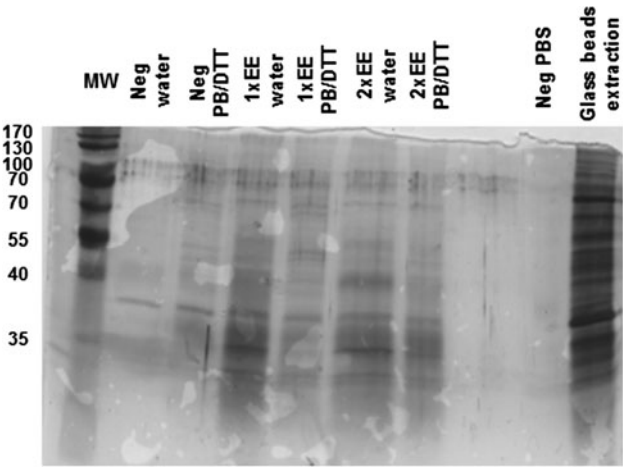
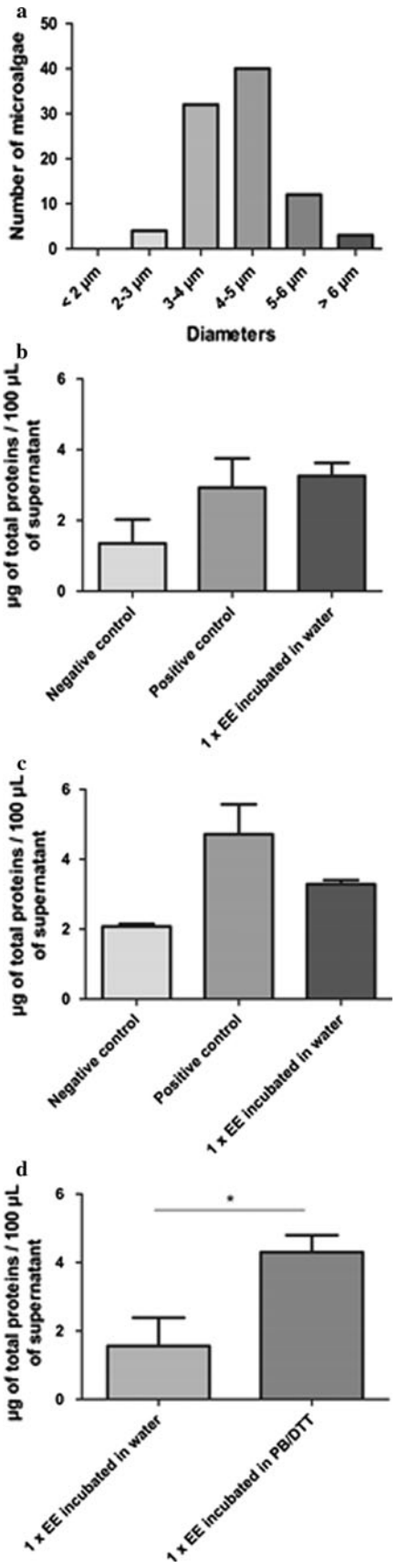


Fig. 8 Electrophoretic analysis of proteins in cell extract and supernatant of electrically treated cells. Protein samples in SDS-PAGE sample buffer containing 2-mercaptoethanol were boiled for 3 min at 90 °C prior to electrophoresis on 12 % acrylamide gel. Gels were stained for protein by silver nitrate



◀**Fig. 9** Total protein assay for *Chlorella*. **a** Size histogram of the population. **b** Negative and positive controls were made with unpulsed suspensions of microalgae, respectively incubated in water or methanol and vortexed. The remaining suspension in water was processed by a 3-kV/cm electroextraction and then incubated either in water or in methanol. The incubation period of all samples and controls was 24 h at room temperature. Concentrations of total proteins were determined by a classical Bradford assay, using BSA as a standard (see "Materials and Methods" section) ($n = 3$). Statistical analyses were made by Prism software using standard error means and a t test. **c** Same as in **b**, but those assays were conducted at 4.5 kV/cm ($n = 2$). Statistical analyses were made by Prism software using standard error means and a t test. **d** Effect of the postpulse extraction buffer (water vs. PB/DTT) ($n = 3$). Statistical analyses were made by Prism software using standard error means and a t test

that the leakage of protein in the negative control, without any treatment applied to the suspension, was rather low. This export of cytoplasmic soluble proteins overnight was also observed in yeast experiments (unpublished results). We could explain this phenomenon by the endogenous secretion of eukaryotic cells and the passive diffusion through the plasma membrane of cytosolic proteins during the incubation period. The electroextraction method, with water as a postpulse incubation medium, improved the recovery of soluble protein by 400 % after overnight incubation compared to the negative control (Fig. 7b). These experiments were conducted six times, and the related results were reproducible.

Those results confirmed that we did permeabilize the plasma membranes of *N. salina*, but as demonstrated on yeasts previously, it induced a structural reorganization of the wall. Cytoplasmic proteins could leak out and be easily recovered.

The possibility for treatment of more concentrated suspensions (from 5×10^7 up to 10^8 cells/ml) was checked. Levels of extraction were two times higher with the higher density in comparison to the lower density (Fig. 7c). But the conductivity of the pulsed suspension was increased with the higher microorganism load. A higher current was delivered, resulting in an increase in the Joule heating and a deformation of the electrical pulse patterns (voltage and current).

The proteins from supernatants of control, electrically treated and mechanically extracted cells were analyzed by SDS-PAGE under reducing conditions (Fig. 8). Most of the bands appeared between 35 and 170 kDa. The same bands appeared on both negative controls and samples, with a higher intensity for the samples incubated in water or phosphate buffer (PB/DTT = PB 105 mM, 0.3 M glycerol, 1 mM DTT, pH 7). Several new proteins appeared after electroextraction in comparison to the negative controls. Most of the proteins recovered by electroextraction were also present in the glass bead extraction.

Protein Extraction from *C. vulgaris*

Microalgae from freshwater were treated under milder conditions (3 kV/cm) due to their larger size (Figs. 7a, 9a). A large level of protein extraction was again obtained (Fig. 9b). These experiments were conducted three times, and the related results were reproducible.

Increasing the field strength did not improve the extraction yield (Fig. 9c).

To limit the Joule heating during the pulse train delivery, the microalgae were in suspension in pure water. One open question is the nature of the incubation buffer where the protein leakage was occurring. In the case of yeasts, we previously showed that optimization was obtained in a high–ionic content buffer with the presence of glycerol and DTT (Ganeva et al. 2003). Reducing agents increased wall porosity by breaking the disulfide bonds in the outer mannoprotein layer. As *Chlorella* is a freshwater microorganism, the question of the need of the ionic postpulsation buffer was open. We compared the relative level of extraction either with pure water or with the medium optimized for yeasts. As shown in Fig. 9d, again a high–ionic content buffer containing DTT brought the higher yield in extraction.

Conclusion

Here, we confirmed on microalgae that flow electroextraction is a highly efficient enzyme extraction technique that allows continuous treatment of large volumes. It permitted efficient protein release from cells from different microalgae genera with small adjustments in electric parameters. Our results suggested that the protocol could be optimized by a simple tuning of just one electric parameter, such as the field intensity in the present study.

According to the theory that the effect of electric pulsation is size-dependent, stronger electric treatment should be applied to obtain the same effect on smaller cells (Neumann et al. 1992; Rols and Teissie 1989; Sixou and Teissié 1990).

It should be underlined that the chosen electrical treatment did not cause cell lysis. This lack of cell fragmentation facilitated the removal of cells from the medium containing the released proteins (centrifugation, coarse filtration). Electroextraction had several advantages if compared with routinely applied methods for intracellular protein recovery. The pulsing (water) and postpulse buffers were cheap. No chemical contaminant was present. This was a very important issue in the European context (Registration, Evaluation, Authorization and Restriction of Chemical Substances, REACH directive EC 1907/2006). The regulation should bring the progressive substitution of the most dangerous chemicals when suitable alternatives have been identified. Our present results provide direct evidence that electroextraction prevented the use of chemical additives for protein extraction. Hazards that they posed to human health and the environment were not present. A single passage through the pulsation chamber was quite enough for inducing the cell envelope alterations necessary for cytoplasmic protein release. Electrical parameters could be preset to limit the heating during pulsation to avoid the use of a cooling system. The system could work continuously. High efficiency could be reached even when leakage occurred at room temperature, which made the method simpler and less expensive. In the case of *Chlorella*, the prepilot designed according to the FP7 "Electroextraction" program was able to treat more than 3 l/h. A larger unit could be easily manufactured (personal communication from Betatech).

Significant extraction was observed on two species growing under highly different conditions but both with rigid cell walls: saline for *Nannochloropsis* and freshwater for *Chlorella*. This told us that there was no effect of the cell wall characteristics on protein electroextractability yield.

One open question was whether irreversible permeabilization of plasma membrane induced a cell wall alteration that increased its porosity and resulted in the leakage of cytoplasmic proteins. This weakening of the interactions was controlled by electrostatic interactions (high–ionic content postpulse buffer) and disulfide bonds (DTT effects) as previously described for yeasts. The direct field effect appeared to be limited to the induction of an irreversible membrane permeabilization.

The process could be applied for recombinant proteins (Walker et al. 2005), especially when they were highly sensitive to proteolytic degradation or treatment with solvents and detergents. This new and innovative technology should soon become a routine user-friendly process in microalgae biotechnology.

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