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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 electrop-ermeabilization 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 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