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Short communication

Aqueous extraction of proteins from microalgae: Effect of different cell disruption methods

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ABSTRACT

The microalgal structure has been investigated to evaluate the release of proteins in aqueous media from five microalgae after conducting different cell disruption techniques: manual grinding, ultrasonication, alkaline treatment, and high-pressure treatment. After conducting cell disruption, the protein concentration in water was determined for all the microalgae and the results are discussed within the context of their cell wall structure. It was found that the aqueous media containing most protein concentration followed the order: high-pressure cell disruption > chemical treatment > ultrasonication > manual grinding. Fragile cell-walled microalgae were mostly attacked according to the following order: *Haematococcus pluvialis* < *Nannochloropsis oculata* < *Chlorella vulgaris* < *Porphyridium cruentum* ≤ *Arthrospira platensis*.

1. Introduction

Microalgae were first exploited for their capacity to accumulate proteins and, through time, interest in this biomass took a new course especially during the last two decades with increasing demand for sustainable energy. This biomass proved to be an important source of lipids suitable for biodiesel production. Hence, many of the studies were concentrated on lipid extraction for fuel purposes, neglecting the potential of microalgae to produce proteins and other high-value components [1]. However, until now all studies and estimates confirmed that costs of production of biodiesel from microalgae remain high [2,3] and far from being competitive with fossil fuel. Researchers are therefore turning towards valuing other components present in the microalgae such as proteins, pigments, dyes, sugars, etc.

Extracting the totality of a specific component from microalgae is often prevented by the intrinsic rigidity of its cell wall. To overcome this barrier, an initial operation unit of cell disruption is required to permit complete access to the internal components and facilitate the extraction process. Hence, many cell disruption techniques have been tested to break the cell wall of microalgae such as bead milling [4,5], ultrasonication [6–8], microwave radiation [9], enzymatic treatment [10,11], cell homogenizer [12] and high-pressure cell disruption [13] to recover different components. The efficiency of cell disruption was usually evaluated by extracting a single component especially lipids before and after applying the treatment or by microscopic observation. To our knowledge, studies of microalgal proteins have been focused on: evaluating the nitrogen to protein conversion factor [14–18]; finding the best method to analyze proteins and differentiate between soluble and non-soluble proteins [19]; and analyzing the behavior of proteins at the air/water interface [20].

Therefore, the present study focuses on evaluating the effect of different cell disruption techniques on protein extractability in water of five different microalgae having different cell wall macrostructures. Namely, the Cyanobacterium *Arthrospira platensis*, which has a relatively fragile cell wall, composed mainly of murein and no cellulose [21,22]. The Chlorophycean *Chlorella vulgaris* and the Eustigmatophyceae *Nannochloropsis oculata*, which have a cell wall mainly composed of cellulose and hemicelluloses [23]. Another Chlorophycean *Haematococcus pluvialis* has a thick trilaminar cell wall composed of cellulose and sporopollenin [12,24,25]. The composition of its cell wall, similar to that of spores, makes this microalga less permeable and extremely resistant to mechanical treatments [26]. Finally, the Rodophythe *Porphyridium cruentum*, which lacks a true cell wall, but instead is encapsulated by a layer of sulfurized polysaccharides [27–32].

In addition, the microalgae selected in this study have a cytoplasm containing soluble proteins, and they all have a chloroplast except for *A. platensis*, which instead has thylakoids bundles circling the peripheral part of the cytoplasm with their associated structures, the phycobilisomes

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(containing the phycobiliproteins) present on the surface of the thylakoids like in the chloroplast of *P. cruentum* [21]. Furthermore, the chloroplast also contains soluble proteins and a central pyrenoid, which is a non-membrane, bound organelle composed of RuBisCO.

In this study, proteins released in the aqueous media were evaluated and discussed considering the cell wall macrostructure of each microalga along with the effect of each cell disruption technique used.

2. Materials and methods

2.1. Microalgae

The microalgae selected are the Cyanobacteria *Arthrospira platensis* (strain PCC 8005), two different Chlorophyceaen *Chlorella vulgaris* (strain SAG 211–19), and *Haematococcus pluvialis* (unknown strain), one Rhodophyta *Porphyridium cruentum* (strain UTEX 161), and the Eustigmatophyceae *Nannochloropsis oculata* (unknown strain).

Each microalga was cultivated in a different culture medium. Hemerick medium was used for *P. cruentum*, Sueoka medium for *C. vulgaris*, Basal medium for *H. pluvialis*, Conway medium for *N. oculata* and Zarrouk medium for *A. platensis*. All strains were grown in batch mode in a 10 L indoor tubular air-lift photo-bioreactor (PBR at 25 °C [33] inoculated from a prior culture in a flat panel air-lift PBR (1 L). Culture mixing was achieved by sterile air injection from the bottom of the PBR. The pH and temperature were recorded by a pH/temperature probe (Mettler Toledo SG 3253 sensor), and monitored by the acquisition software LabVIEW. The pH was regulated at 7.5 with CO_2 bubbling. Microalgae were harvested during the exponential growth phase and concentrated by centrifugation, and then supplied as frozen paste from Alpha Biotech (Asserac, France). The biomass concentration of the paste was 20–24% dry weight.

2.2. Reagents

The Lowry kit (a prepared mixture of Lowry reagent, BSA standards and 2 N Folin-Ciocalteu reagents) was from Thermo Scientific. NaOH granules and 37% HCl were purchased from Sigma Aldrich and used as received.

2.3. Microalgae pre-treatment

2.3.1. Freeze-drying

The frozen paste of crude microalga (about 70 g) was directly introduced to a Fisher Bioblock Scientific Alpha 2–4 LD Plus device (Illkirch, France). The pressure was reduced to 0.010 bar and the temperature was further decreased to −80 °C and freeze-drying was conducted under vacuum for 48 h. Dry biomass was stored under anhydrous conditions. Before any disruption treatment, the cells were vigorously rehydrated in distilled water to ensure good homogeneity of the sample.

2.4. Microalgae treatments

2.4.1. Control

Cells (0.5 g) were dispersed for 2 h in 25 mL distilled water and the supernatant was recovered by centrifugation at 10,000 *g* for 10 min at 20 °C for protein analysis. This treatment was considered as a blank to compare with the other extraction treatments.

2.4.2. High-pressure cell disruptor

A TS Haiva series, 2.2-kW, disrupter from Constant Systems Limited (Northants, UK), was applied, in two passes at a pressure of 2700 bar, to a biomass sample at a concentration of 2% dry weight (0.5 g of dry cells dispersed in 25 mL distilled water).

2.4.3. Ultrasonication

This treatment was carried out using a VC-750HV (20 kHz, 13 mm probe) ultrasonic processor on 0.5 g of dry cells dispersed in 25 mL distilled water. Total treatment time was 30 min in cycles of 5 s of ultrasonication and 15 s of resting time in order to prevent overheating the sample.

2.4.4. Manual grinding

Dry microalgae were manually ground using a mortar for 5 min, and then 0.5 g was dispersed in 25 mL distilled water for 2 h. Samples were taken for protein analysis.

2.4.5. Chemical treatment

Mother solutions were prepared with approximately 500 mL of distilled water and 2 N NaOH was added to adjust the solution to pH 12 for maximum protein solubility. A sample of 0.5 g of freeze-dried biomass was added to 25 mL of mother solution. The mixture was then stirred for 2 h at 40 °C. The separation of the supernatant from the pellet was conducted by centrifugation at 10,000 *g* for 10 min at 20 °C. The supernatant was then adjusted to pH 3 with 0.1 M HCl in order to precipitate the proteins. The protein isolate was collected after centrifugation at 10,000 *g* for 10 min at 20 °C and the pellet was neutralized with 0.01 M NaOH [20]. Samples were taken for protein analysis.

2.5. Lowry method

After every disruption treatment, the liquid/solid separation was conducted by centrifugation at 10,000 *g* for 10 min at room temperature and the supernatant was analyzed by the Lowry method. [34]

A calibration curve was prepared using bovine standard albumin at a concentration range of 0 to 1500 μg mL^{-1}. In order to measure the protein content, 0.2 mL of each standard or samples containing the crude protein extract was withdrawn and then 1 mL of modified Lowry reagent was added to each sample. Each sample was then vortexed and incubated for 10 min. After incubation, 100 μL of Folin-Ciocalteu Reagent (1 N) was added and again vortexed and incubated for 30 min. The blue color solution was then measured at 750 nm with a UV-1800 Shimadzu spectrophotometer.

2.6. Elemental analysis

Total nitrogen was evaluated by using a Perkin Elmer 2400 series II elemental analyzer. Microalgal samples (2 mg) were placed in tin capsules and heated at 925 °C, using pure oxygen as the combustion gas and pure helium as the carrier gas, and the nitrogen concentration was evaluated. For all the previous analyses, three experiments were conducted separately with all the microalgae.

2.7. Confocal laser scanning microscopy

Cells were observed with an SP2-AOBS confocal laser-scanning microscope from Leica microsystems (Nanterre-France). The fluorochrome calcofluor white that binds to the cell wall was added to the samples. When excited at 488 nm, the cells are identified as light blue colored.

2.8. Statistical analysis

Three experiments were conducted separately on all microalgae and their protein extract. Statistical analyses were carried out on Microsoft Excel 2011 and Statgraphics Sigma Express. ANOVA test was carried out and measurements of three replicates for each sample were reproducible for ±5% of the respective mean values.