Food and Bioprocess Technology

Supplementary Information

High purity grade phycocyanin recovery by decupling cell lysis from the pigment extraction – an innovative approach

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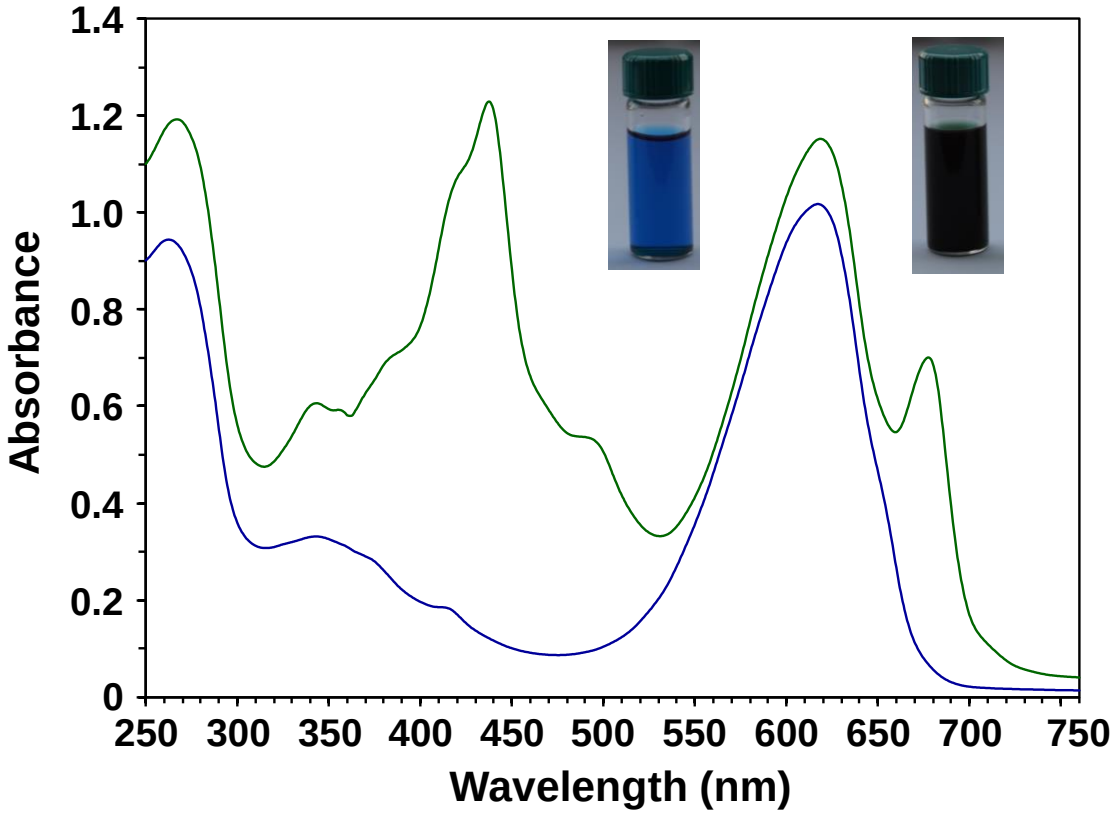

Fig. S1 Absorbance spectra of PC crude extract solutions (NaCl 0.1 M) obtained following Spirulina cells breaking by freeze-thawing (1, blue curve) and by ultrasonication (2, green curve) procedures, respectively. Insets: PC extract solutions obtained by freeze-thawing (blue extract) and by ultrasonication (green extract) procedures

Determination of biomass stock suspension dry weight (d.w.)

In order to operate under similar conditions of extracting solution volume (μL)/biomass (mg, d.w.) ratio along the various sets of experiments, the dry weight of each biomass stock suspension (mg mL^{-1}) was approximately assessed by measuring the optical density (OD) at 560 nm (Eq. **(1S)**).

$$\text{d.w. (mg mL}^{-1}\text{)} = 0.43 \times OD_{560} \quad \textbf{(1S)}$$

In particular, the spectrophotometric determination was performed on a diluted aliquot of the stock suspension (usually 0.1 mL of stock suspension was added to 3 mL of Milli-Q water) and then the dry weight of the stock calculated. Suprasil quartz cuvettes with an optical path of 1 cm were used. The biomass dry weight of each stock suspension of *A. platensis* was also determined by drying in a small aluminium tray usually 5 mL of stock suspension, at 105 °C for 6 hours. The value obtained with this procedure was that used in the calculation of both, the pigment yield and the volume/biomass ratio. The safe use of aluminium trays was evaluated by performing some dry weight determinations using both aluminium and glass trays. It was not possible to use glass microfiber filters to determine the biomass dry weight, due to high density of biomass stock suspension. In fact, the stock suspension d.w. was around 10 mg mL^{-1} or even higher. In particular, the experiments executed at low volume/biomass ratio required a very high biomass density, around 37 mg mL^{-1}.

One-step conventional ultrasound-assisted extraction: evaluation of cell lysis and PC extraction by direct ultrasonication in the extracting solution to set up the control extraction procedure

All the ultrasonication procedures were performed with a Hielscher Ultrasonic Processor UP200S (200 W, 24 kHz) using an S2 sonotrode. $CaCl_2$ 0.1 M was used as extracting solution in the one-step control standard extraction procedure. In brief, the appropriate volumes of Milli-Q water, $CaCl_2$ 1 M and biomass stock suspension were mixed in 15 mL conical centrifuge tubes (polypropylene) in order to have 5 mL of suspension in $CaCl_2$ 0.1 M at the designed volume (μL)/biomass (mg, d.w.) ratio (R). During the ultrasonication the samples were maintained in water/ice to prevent phycobiliprotein degradation. They were sonicated for several time intervals: 2 min cycles at 100 % power, pulse 1 s (i.e., in continuous mode), with at least 1 min pause in water/ice between two consecutive ultrasonication cycles. Following ultrasonication, the samples were shaken (300 rpm) for some time (extraction time) at ambient temperature by an orbital shaker and centrifuged (30 min, 12000 x g, 15 °C). The supernatants (crude extracts) were recovered and phycobiliprotein content and PC purity determined by spectrophotometric analysis. Several sets of different duration of ultrasonication and extraction were evaluated. The effect of biomass density on the ultrasonication efficiency was also investigated using two different volume/biomass ratios: $R \approx 1200$ ($R = 1213 \pm 13$) and $R \approx 250$ ($R = 244 \pm 19$).

Protocol of Cell lysis/purification phase procedures

The following Cell lysis/purification phase procedures were adopted in order to study the effect of (a) AS concentration and (b) the number (one (b1) or two (b2)) of lysis/purification cycles:

(a) *effect of ammonium sulphate concentration* –

Appropriate volumes of Milli-Q water, biomass stock suspension and AS 3 M were mixed in 15 mL conical centrifuge tubes (polypropylene) to have 5 mL of suspension at the designed AS concentration (0.4 – 1.5 M). AS was always added as last component. The suspensions were subjected to the one-cycle lysis/purification procedure (b1) as described below.

(b) *effect of the number of cycles in lysis/purification phase*, one-cycle (b1) and two-cycle (b2) procedures –

In the <u>one-cycle procedure (b1)</u> the samples (biomass suspensions in AS cleaning solution) were ultrasonicated four times for 2 min (100 % power, continuous mode), in water/ice bath, with at least 1 min pause (in water/ice bath) between two consecutive ultrasonication cycles to avoid phycobiliprotein degradation. After ultrasonication, samples were shaken (300 rpm) for 10 min at ambient temperature by an orbital shaker and centrifuged (15 min, 12000 x g, 15 °C). The supernatants were discarded while the biomass pellets were recovered for the following phycobiliprotein extraction procedure.

In the <u>two-cycle procedure (b2)</u>, a cleaning solution with AS concentration of 1.1 M was used. Again, the appropriate volumes of MilliQ water, biomass stock suspension and AS 3 M were mixed in 15 mL conical centrifuge tubes (polypropylene) to have 5 mL of suspension in AS 1.1 M. The samples were ultrasonicated two times for 2 min (100 % power, continuous mode), in water/ice bath, with at least 1 min pause (in water/ice bath) between the two consecutive ultrasonication cycles to avoid phycobiliprotein degradation. Following ultrasonication, the samples were shaken (300 rpm) for 5 min at ambient

temperature (20-24 °C) by an orbital shaker and centrifuged (15 min, 12000 x g, 15 °C). The supernatants were discarded, the biomass pellets were suspended in 5 mL AS 1.1 M and the ultrasonication treatment, followed by centrifugation, repeated a second time, as described above. The supernatants were discarded while the biomass pellets were recovered for the following phycobiliprotein extraction procedure.

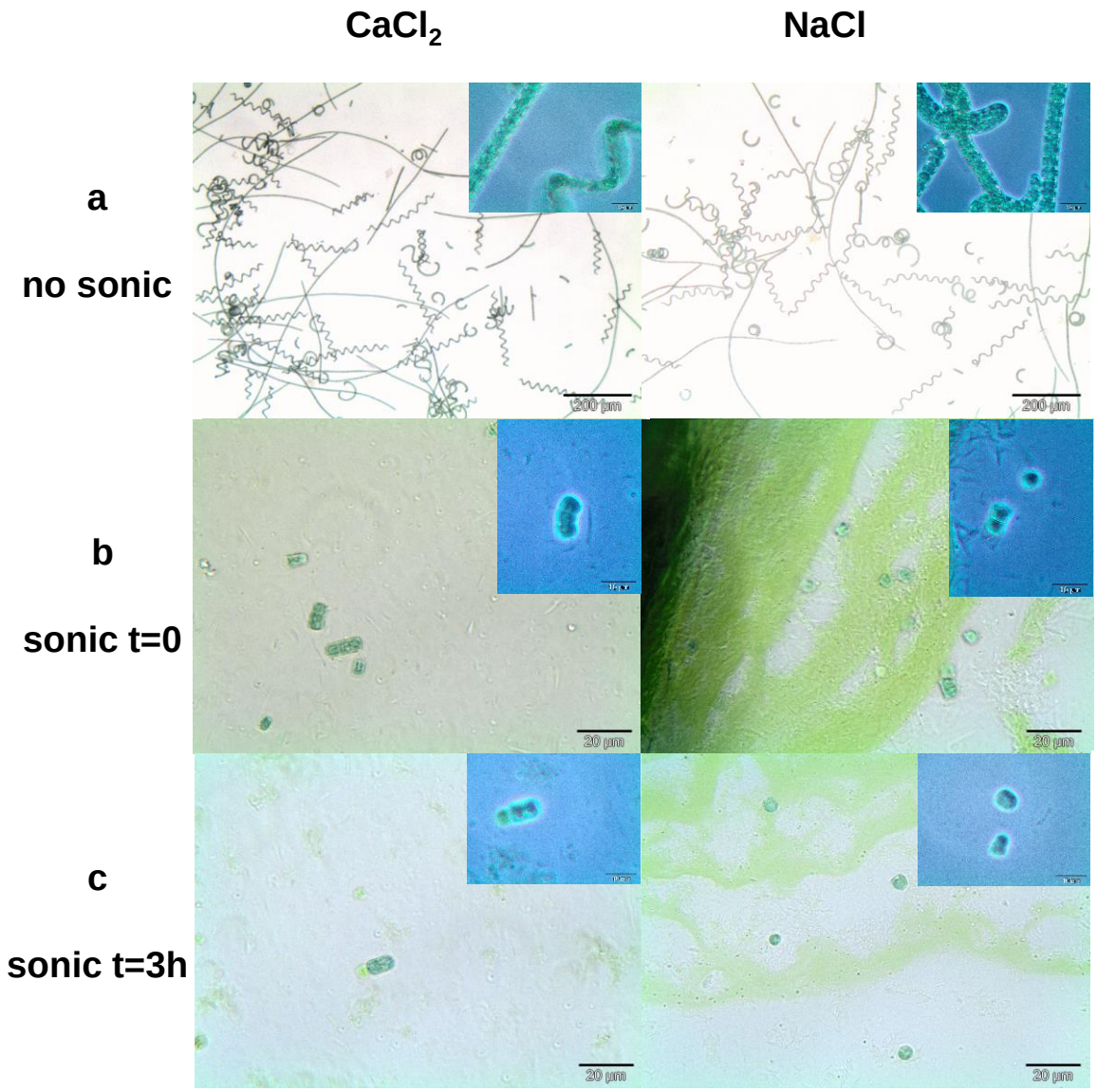

Fig. S2 Optical microscope images of biomass suspensions in $CaCl_2$ 0.1 M and NaCl 0.1 M, before and after ultrasonication (soon after ultrasonication and after 3 hours). Suspensions were observed with a Zeiss Axiolab light microscope (dark field, phase contrast) (Gottingen, Germany) and photographed with an Olympus SC30 Digital Camera (Münster, Germany). (a) magnification 5x; scale bar 200 µm. (b-c) magnification 40x; scale bar 20 µm. Inset images magnification 100x; scale bar 10 µm

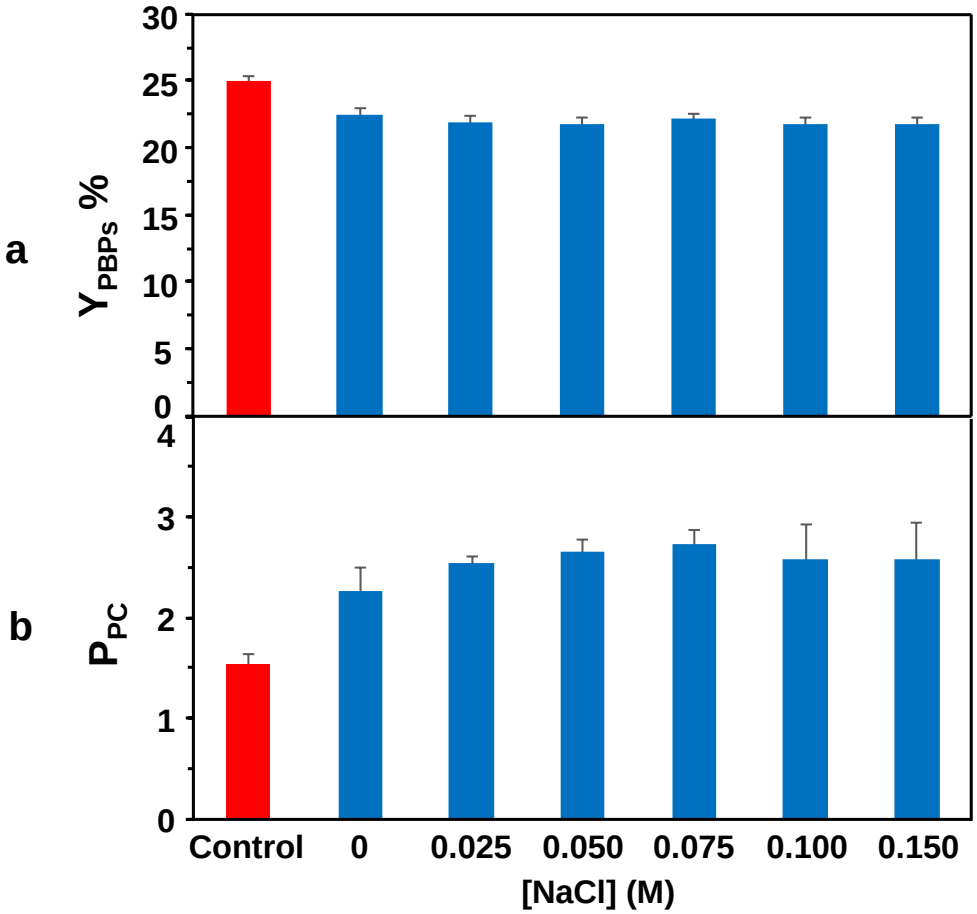

Fig. S3 Evaluation of the effect of NaCl concentration. The one-cycle lysis/purification (b1) procedure was applied. (a) Yield % of total extracted phycobiliproteins (PC + APC) (Y_{PBPs}%); (b) PC purity grade (P_{PC}). Data are the average of 4 independent experiments using two different biomass batches