Breakthrough Technology for the Production of High-Value Nutraceutical Products and Biofuels from Microalgae

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In recent years, microalgae have been shown to be a highly promising feedstock for biofuels and high-value bioproducts (e.g. chlorophyll, β-carotene, ω-3 oils and protein) because of their high productivity and non-requirement for agricultural resources (e.g. arable land and freshwater for marine microalgae). These products are trapped inside the cells and can generally only be made available for further processing after they have been liberated from the cell walls. Microalgal cell walls, however, are composed of tough interlocking sugar and protein chains that confer the cells with a formidable defence. The high energy cost associated with disrupting the cells to liberate bioproducts has remained a major barrier to the commercial production of microalgal bio-industry.

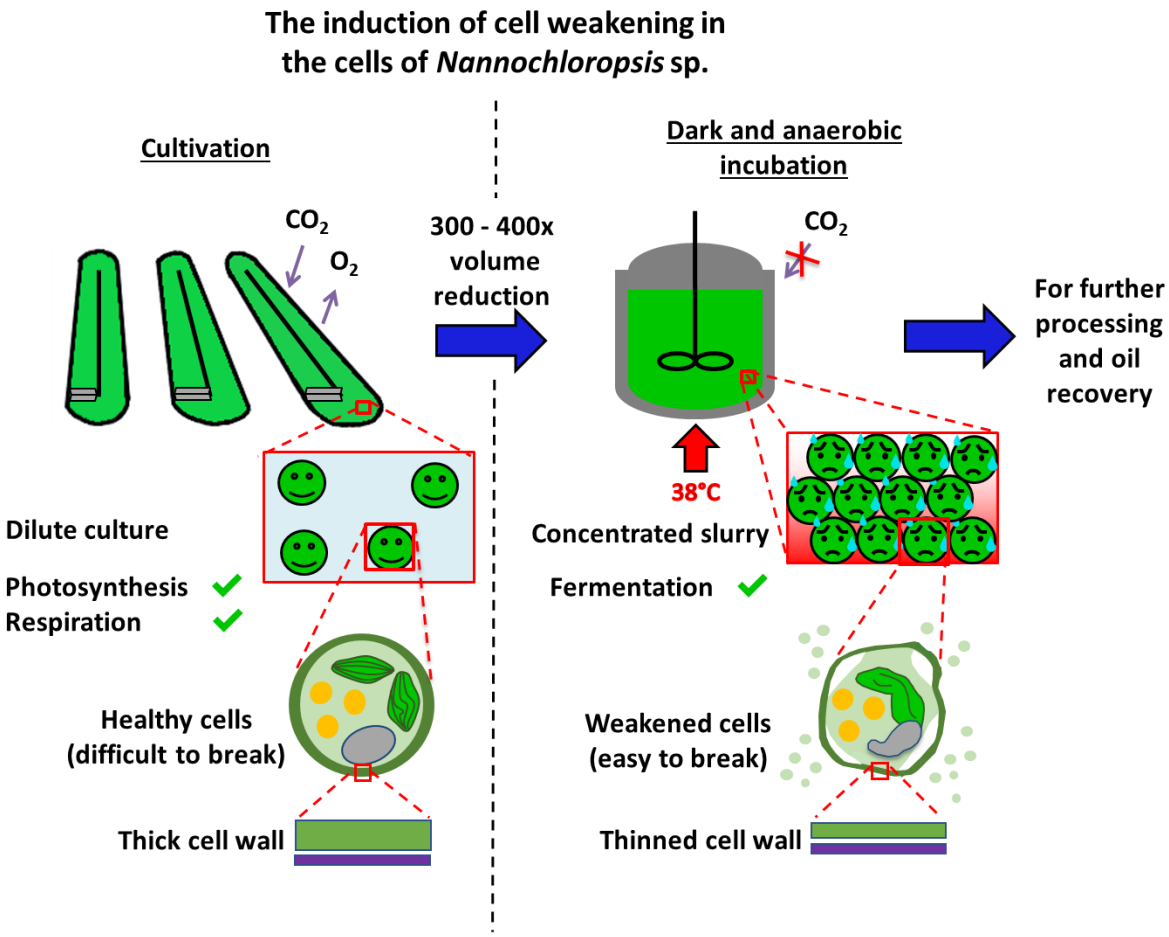
In this study, we present a simple and low-cost incubation treatment able to induce cell-wall self-degradation in wet *Nannochloropsis* cells and enhance bioproduct extraction. *Nannochloropsis* sp. is an industrially promising group of saltwater microalgae strains with high contamination resistance and biofuel-convertible oil content (up to 60 wt% of the biomass). Under our dark and anaerobic (no-oxygen) incubation treatment, *Nannochloropsis* cells were packed tightly inside a dark container and agitated at 38°C and 60 rpm for 16 - 24 h. During this incubation, the cells stopped photosynthesizing, ran out of oxygen and activated a fermentation metabolism that consumed part of their own cell walls to survive. The fermentation process reduced the thickness of the cell walls by ~50% and made the cells more fragile to disruption.

The treatment appeared to be robust, being able to consistently inflict cell weakening in three different *Nannochloropsis* strains, a *Nannochloropsis* strain grown under different conditions (nutrient-rich and nutrient-poor) and a *Nannochloropsis* strain being processed with different disruption methods (mechanical disruption with high pressure or chemical disruption with acid and alkali). No oil or fatty acid degradation in the biomass was observed throughout the incubation treatment.

When implemented as part of a *Nannochloropsis* biorefinery system, the treatment increased the level of cell rupture from ~37 to ~74 % of available cells and doubled the extraction yield of biofuel-convertible oil from 22 to 49 wt% of available oil. The treatment has also been shown to be able to enhance the extraction yields of ω-3 oils and proteins from *Nannochloropsis* cells.

Our evaluation demonstrated that the energy cost of the treatment was minimal and that its implementation would lead to a net positive energy balance for the biorefinery system. The application of the treatment to other industrially relevant microalgae strains (such as *Chlorella* sp. and *Haematococcus pluvialis*) will depend on the strains' ability to induce cell wall degradation.

Overall, our dark anaerobic incubation was shown to be a scalable treatment that can be readily integrated in a *Nannochloropsis* biorefinery system to enhance biomass processing and increase bioproduct yields. The treatment has significant potential in reducing the energy requirements for microalgal biomass processing and facilitating the development of an energy-efficient (and thus cost-effective) microalgal biorefinery system.


The induction of cell weakening in the cells of *Nannochloropsis* sp.
Cultivation
CO_2
O_2
300 - 400x volume reduction
Dark and anaerobic incubation
CO_2
For further processing and oil recovery
38°C
Dilute culture
Photosynthesis
Respiration
Concentrated slurry
Fermentation
Healthy cells (difficult to break)
Thick cell wall
Weakened cells (easy to break)
Thinned cell wall