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TOWARDS SUSTAINABLE MICROALGAL BIOREFINERY: INDUCTION OF CELL-WALL SELF-INGESTION IN MICROALGAL CELLS TO REDUCE ENERGY REQUIREMENT FOR BIOMASS PROCESSING

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We present thermally coupled dark-anoxia incubation as a novel and low-energy treatment for inducing autolytic cell wall thinning in microalgal cells. Using this method, we incubated highly concentrated paste (25 wt% solids) of lipid-rich *Nannochloropsis* cells in darkness at 38°C for 24 hr. The treatment halved the thickness of microalgal cell wall (from 66.9±14.8 nm to 33.6±14.5 nm) and, as a result, increased the susceptibility of the cells to mechanical rupture. High-pressure homogenisation (1100 bar) of the paste after incubation was able to rupture more than 70% of the available cells (the same homogenization applied to paste that had not been incubated was only able to rupture ~35% of the available cells)

Under the treatment, *Nannochloropsis* cells activated fermentative pathways that consumed intracellular sugar, in particular cellulose in the cell wall (hence the cell-wall thinning effect). During the treatment, we observed a reduction in the total cellular sugar content (from 13.17 to 6.58 wt% of biomass) and an increased secretion of organic acids as fermentation products (from 0.0008 to 0.0228 g/g biomass). The fermentative pathways, however, did not degrade any of the cellular lipid storage. Detailed lipid analysis of the cells before and after incubation showed negligible change in the total transesterifiable lipid and EPA contents of the cells, respectively at 20.7±5.9 and 2.9±0.4 wt% of biomass.

Thermally coupled dark-anoxia incubation is an attractive pre-treatment step to be implemented in microalgal biorefinery for the co-production of biofuels and high-value bioproducts. The treatment utilizes microalgal cells' biologically driven self-lysing properties to thin their own cell wall, and thus reduce energy requirements for biomass processing, without introducing any decomposition to biofuel-convertible lipid components.

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