**Microalgal biorefinery approach: integration and co-optimisation of the different unit operations.**

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**Highlights**

* Highest energetic performances of bead milling are for inlet cell concentration ≥ 50 g/L
* Separation and purification steps are negatively affected by the optimal BM parameters
* Integrated optimization is required as well as techno-economic analysis.

**1. Introduction**

In the last years microalgae production attracted rising interest and a peak of governmental and industrial investment mainly aimed at green and sustainable food/feed and pharmaceutics production. Large segment of the current market involves the production of raw and entire biomass and only few high-value products extractions. One of the issues limiting the use of algae feedstock for several industrial fields (food, beverages, bio-plastics and bio-materials, cosmetics) is the lack of optimized downstream technology for microalgae components fractionation at industrial scale [1]. In this study, the fractionation of soluble and not soluble product is investigated for the widely recognized microalga *Chlorella vulgaris*. In particular, bead milling is used as cell disruption technology then, centrifugation and membrane filtration are applied for water soluble product separation and purification.

**2. Methods**

Microalgal strain

The microalgal strain used in this study was *Chlorella vulgaris*. The biomass was gently provided by MIAL (Germany) in the form of paste 20% dry matter.

Bead milling cell disruption

Disruption experiments were performed by bead milling (*Dyno-mill multi lab*) operated in pendulum mode, at temperature of 20°C, flow rate of 200 mL min-1 and filling ratio of 80%. Beads density, beads diameters, the rotation speed and initial biomass concentration were varied in order to test different stress intensities. Specific energy requirement for the 90% of cell disruption was calculated according to Montalescot et al. [2]:

Centrifugation

Centrifugation parameters (speed, g and time, min) were chosen in order to meet the availability in the existing large-scale facility of GEPEA (Algosolis): 8000 g for 20 min.

Membrane filtration

The filtration process was conducted with a pilot scale filtration plant. All membranes used (0.22 µm and 3 kDa) were made of ceramic to minimize interactions between the compounds and the membrane. The transmembrane pressure (TMP) was arbitrary fixed at 2 bar for 0.22 µm membrane and 5 bar for 3 kDa which corresponds to classical transmembrane pressures for such operation. The recirculation flow rate was fixed at 250 L h-1.

Biochemical analysis

The entire biomass, the solid and liquid fractions obtained from the different downstream steps were analysed for proteins, pigments, sugars and lipids content according to AOAC methods.

Technoeconomic analysis

A complete biorefinery model was proposed for the adopted strain. The techno-economic feasibility of the process was tested using the software SuperPro Designer (Intelligen Inc.).

**3. Results and discussion**

The combination of bead milling/centrifugation/membrane filtration was analyzed for *Chlorella vulgaris*. Optimal bead milling parameters (0.5 mm glass beads, filling ratio 80%, rotational speed of 8 m/s) and biomass concentration (150 g/L) were identified for soluble proteins release in the water fraction and for low energy consumption related to disruption step. Theoretical and experimental studies showed the impossibility of processing the lysate obtained if the optimization is solely done on bead milling. Indeed, the following centrifugation at large scale produced a liquid fraction characterized by intense green colour, high viscosity, presence of numerous small particles. The nature of the liquid fraction also prevented the exploitation of membrane filtration as solvent-free purification technology. A new refining model was proposed and validated by techno-economic analysis.

**4. Conclusions**

The optimal yield and energetic performances of a single downstream step (bead milling) rarely produce the optimal raw material for the following purification or transformation steps. Different solutions have been proposed and the techno-economic feasibility of the new bio-refinery process was proved.

**References**

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