**BIOCOMPATIBLE EXTRACTION OF β-CAROTENE FROM *DUNALIELLA SALINA –* NEW CONTRIBUTION**

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**Highlights**

* The extraction is biocompatible at the culture level but not at the cell level
* The biocompatibility of the extraction follows a granulo-selective phenomenon
* The extraction kinetics were obtained using Centrifugal Partition Chromatography

**1. Introduction**

Biotechnological production of carotenoids from the green microalga *Dunaliella salina* involves two steps: (i) biomass growth followed by β-carotene accumulation inside the cell and (ii) carotenoid extraction. Microalgae milking was introduced for the recovery of biomolecules directly from culture broth using long-chain alkanes as solvent [1]. In this condition, photosynthesis was not highly impacted [2] and the fraction of extracted β-carotene from biomass exceeded the fraction of disrupted cells [3]. In a biocompatible extraction or in situ extraction concept, cells remain alive during extraction and the extracted molecules can be re-accumulate [1].

The aim of this study was to determine how the extraction of β-carotene from the green microalga *Dunaliella salina* was biocompatible. The solvent used was *n*-decane and two extraction parameters were investigated: (i) time of contact and (ii) interfacial area between the solvent and the microalgal culture.

**2. Methods**

*Dunaliella salina* was grown in a 1L Air-Lift flat panel Photobioreactor (PBR) [4]. To trigger β-carotene accumulation, the green biomass was stressed through a combination of nitrogen limitations and light stress (400 µE/m²/s) or, nitrogen depletion and high light stress (800 µE/m²/s). After carotenoid accumulation, the extraction was run on a Centrifugal Partition Chromatography (CPC), which allowed to control the two liquids phases hydrodynamics with centrifugal acceleration [5]. After extraction the biomass was re-inoculated within the same medium or in the growth medium. The biomass was analyzed before and after extraction and during the re-growth to access the biocompatibility and kinetics of relaxation. Analysis were focused on cell granulometry (density and size), metabolites (pigments and lipids) and dry biomass regarding the extraction parameters.

**3. Results and discussion**

As a first result, total cell volume decreased as a function of time of contact of the culture media and the solvent (Figure 1). Concerning the cell volume distribution before and after extraction, the biocompatibility is lower for big than for small cells (“granulo-selective” phenomenon) and the median value µ of the distribution decrease (*p* < 0.05). As a consequence, difference between population biocompatibility and individual biocompatibility appeared a key point and a new contribution. These results suggest that carotenoid and lipid extraction yields were correlated with cell destruction.

Finally, a comparison has been made between the two stress applied to the biomass and the scenarii of re-growth to determine the most productive process scheme.



**Figure 1.** Cell volume distribution (Left) and light microscopy pictures for different extraction time (Right). The larger the extraction time the shorter the mean median value and the fewer cells survive. **a)** Untreated, **b)** 45 s, **c)** 120 s and **d)** 240 s of contact between the solvent and the microalgal culture. Cells were fixed with 1% lugol.

**4. Conclusions**

Centrifugal Partition Chromatography allowed the decoupling of biomass/metabolites production and the extraction steps giving a better understanding of the mechanism. The granulo-selective effect of the solvent extraction is an original contribution that opens the reflexion for new carotenoid production schemes. The scenarii investigated allowed to choose suitable conditions for the open-loop process with high metabolite productivity and low solvent consumption.

**References**

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