SCALING-UP OF A B-PHYCOERYTHRIN PRODUCTION AND PURIFICATION BIOPROCESS INVOLVING AQUEOUS TWO-PHASE SYSTEMS: PRACTICAL EXPERIENCES

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Introduction. One of the most attractive segments in food and cosmetic industries is that of natural pigments. Since some synthetic pigments have been reported to be hazardous for humans, natural pigments obtained through biotechnological processes represent an attractive alternative (1). Our research group has previously developed a bioprocess for the selective production and recovery of highly purified (high purity defined as the absorbance ratio A_{545}/A_{280}>4) B-Phycoerythrin (BPE) with an aqueous two-phase system (ATPS) platform (2).
The objective of this research was to determine the technical feasibility of the scaling-up of a BPE production and purification bioprocess using *Porphyridium cruentum* as expression system.

Methods. The proposed scaled-up process comprised a 250 L photobioreactor for the generation of *P. cruentum* biomass, a cell disruption stage in a continuous bead mill, a pre-fractionation and concentrating step using isoelectric precipitation at pH 4.0, a liquid-liquid fractionation using PEG – potassium phosphate ATPS, and an ultrafiltration stage for polymer removal and final concentration (Fig.1). Process parameters at pilot-plant scale were characterized and optimized in order to maximize overall yield without compromising purity.

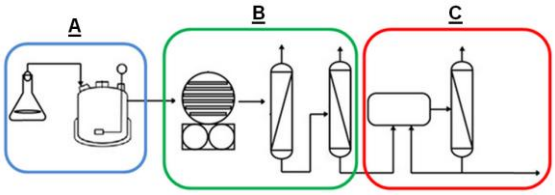

Fig 1. A) 250 L photobioreactor, B) cell disruption of biomass, precipitation and ATPS purification, C) ultrafiltration polishing step

BPE purity and concentration in each stage were determined using a previously reported equation system based on the constant extinction coefficients of R-phycocyanin, Allophycocyanin and BPE at 565, 620 and 650 nm respectively (3).

Results. The characterization of the parameters of each stage allowed the optimization of the previously developed lab-scale bioprocess. An overall recovery yield of 55% and a purity > 4 for BPE were achieved. Table 1 presents the yields and BPE purity at each stage of the scaled process. Differences on yield between pilot-plant and lab scale (~70%) processes were attributed to centrifuge related operations, particularly isoelectric precipitation.

Table 1. Recovery and Purification of BPE on the scaled-up bioprocess

Operation	BPE Purity (A545/A280)	Stage BPE recovery (%)	Overall BPE Recovery (%)
Reactor	0.98±0.03	-	-
Bead Mill	0.98±0.03	100%	100%
Isoelectric precipitation	1.76±0.22	65%	65%
ATPS	4.1±0.08	85%	55%
Ultrafiltration	4.1±0.11	100%	55%

Conclusions. The technical feasibility of the scaling-up of a previously bioprocess for the production and purification of BPE was demonstrated. The proposed process offered simplicity and scalability. The production cost for BPE with the proposed bioprocess was estimated to be ~$1.2 USD/mg BPE, while the commercial price of the product is superior to $30 USD/mg BPE.

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