Intenso Newsletter 4/2017

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Editorial:

Dear reader, this is the last newsletter of the project Intenso as the project will come to end with the end of January 2017. Good progress was made during the research period and we like to present you a short overview of the achievements. Also, we would like to invite you to the first "BioIntenso Inaugural Conference" that will take place on January 24th in Brussels**.** Please find detailed information below.

Results of the Aqueous Two Phase Extraction (ATPS) method

The work has continued with the development and optimization of protein extraction methods from microbial cell cultures, based on irreversible plasma membrane permeabilization induced by pulsed electric field (PEF), the main goal being selective recovery of recombinant and native proteins from different yeast species, as an alternative to mechanical disintegration, avoiding, in addition, cell debris generation. By applying PEF in continuous flow chambers, in combination with a treatment with small amounts of lytic enzymes, suitability and scalability of the process was demonstrated by recovery of different proteins including recombinant human ferritin from Hansenula polymorpha (up to 90%, purification factor 1,8), beta-galactosidase from Kluyveromyces lactis (80%, purification factor 2,6), recombinant lipoxygenase and a fluorescent protein from Pichia pastoris. Preliminary experiments show that PEF can be specially useful in selective extraction of protease inhibitors from baker's and brewer's yeast, as well as from H. polymorpha, Pichia membranifacies and K. lactis. Dovetailed, downstream processing of PEF extracted proteins has been explored by aqueous two-phase extraction (ATPE), with preliminary results showing efficient separation of electroporated cells. ATPE procedures have been applied also to other bioproducts studied in the project, including virus-like particles (VLP) and recombinant proteins fused to Zera®, a self assembler peptide that allows recovery of any protein of interest as a protein body, with enhanced immunogenicity in the special case of vaccines. ATPE of secreted Zera®-fused proteins, developed based on their predicted partitioning behavior, allowed their recovery from culture media with significant enrichment and compatibility with downstream induction of Zera®-driven aggregation into protein bodies, introducing a cost-efficient strategy for the processing of Zera®-fused proteins as an alternative to previously developed, chromatography-based procedures. Another protein purification technology, based on the combination of the choline-binding tag LYTAG, with a self- excisable intein, has been developed. With this method, any protein or polypeptide can be expressed in Escherichia coli as a fusion to its C terminus of an Intein-LYTAG double module, captured in a Q-anion exchanger (a choline structural mimetic), extensively washed with high ionic force and recovered (> 90%) in the mobile phase, with high purity (> 90%), by induction of intein-promoted cleavage with a reducing agent. LYTAG has also been successfully applied to the purification of monoclonal antibodies (mAbs) by ATPE using a recombinant, dual ligand named LYTAG-1xZ (produced with E. coli in high-density cultures), with two separate domains exhibiting affinity for polyethylene glycol (PEG, another choline structural mimic) and the IgG constant region of mAbs, respectively. In a PEG/Dextran - based ATPE, LYTAG-1xZ allowed affinity-driven partitioning of the mAbs to the PEG-rich phase, resulting in 89% recovery with 42% purity, and complete cell separation, when tested with 4 kg of cell culture load. A downstream polishing step, based on LYTAG-mediated affinity to a Q-fiber cartridge, allowed capture and sequential elution of the mAbs (at a relatively mild pH value of 3,8, resulting in 81% recovery and 89% purity), and the LYTAG-1xZ polypeptide (using the LYTAG specific ligand, choline), which could be recycled in the process. Finally, continuous phase separation in ATPE has been explored by adapting the hydrocyclone technology. A first prototype has been developed by combining variations

in different parameters including vortex finder, cylinder and underflow diameters. Additional optimization will be required for the system to work with the required sample loads.

Results of the Convective Flow Systems (CFS) method

The main objective was to evaluate the performance of convective flow systems (CFS) in capturing and purifying large biological molecules (plasmid DNA – pDNA, virus like particles - VLPs, and messenger RNA – mRNA) with tremendous potential as biopharmaceuticals of the future. The results of the work contribute to the development and implementation of CFS in industrial downstream processing practice especially due to more efficient and cost effective (bio) manufacturing.

We have shown during the project that CFSs are almost ideal for purification of very large biological molecules in terms of separation power, capacity, and recovery and molecule integrity. Radial chromatography enables scale up of chromatographic monoliths to industrial scale without compromising the speed of operation or separation efficiency. Additionally, we have developed a hybrid TFF/monolithic adsorber cartridge – BiAxcys module, enabling crude feed processing with standard CIM chromatographic monoliths monolith cartridges. The BiAxcys prototype II is capable of purification from crude feed, bypassing all industry standard clarification steps. This ability has great advantage in use of; power, reagents, consumables and processing time.

Using chromatographic monoliths in radial flow configuration we have reached improvements in downstream processing of:

- virus like particles (development of two orthogonal methods for purification of VLP directly from the clarified culture supernatant);

- messenger RNA (development of completely new chromatographic purification method employing hydrophobic interaction chromatography using CIM monoliths);

- plasmid DNA (upgrade of existing chromatographic methods with sample displacement chromatography in order to achieve higher productivity; development of new generation of chromatographic monoliths for plasmids ranging above 10 kbp).

“BioIntenso Inaugural Conference”

To exploit and sustain the project results the consortium organised “BioIntenso”. BioIntenso is an open interest group for downstream processing (DSP). It serves as a forum and meeting place for people working in and with DSP technologies. The first BioIntenso Inaugural Conference will take place on January 24th, 2017 in Brussels. Detailed information about this event can be found on our project website or on: http://biointenso.com.

More information about the project can be found on our website: http://intensoproject.eu/