Intenso Newsletter 1/2014

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Editorial:

The project “Intenso - Gaining Productivity, Cost Efficiency and Sustainability in the Downstream Processing of Bio Products by Novel Integration and Intensification Strategies” funded by the European Commission and planned with a duration of 54 months with 16 partners from 11 countries.

The Intenso project is about identifying the bottlenecks of the currently used downstream processing methods, and finding new solutions to circumvent them. This is highly relevant for any and all biotechnical products, as downstream processes account for more than 80% of the total manufacturing costs. The Intenso approach will not concentrate solely on the effectiveness of the new methods, but will strive to lead to a sustainable approach, factoring in risk management derived from economic, environmental, and social developments, also including government policies and legal constraints. Intenso proposes an integration / intensification strategy resting on four technological pillars, targeting innovative new technologies, coupled with horizontal activities such as impact assessment, demonstration, training and dissemination. The results of Intenso are expected to expand the current industrial downstream processing practices significantly, achieved by studying various technologies and utilizing a nano-to-process strategy, so as to introduce integration / intensification during bioprocesses.

With this first newsletter we would like to introduce you more deeply to our project work and the different tasks of our partners, starting with a short overview of the partner Generi Biotech’s part in the project.

What is the challenge for Generi Biotech working within the Intenso project?

Generi Biotech was interested in this project from the very beginning, because the purification of biological material is as important as the production. For the future, it is highly important to produce samples of very high purity with good recovery from different biological sources. Our company is specialized in upstream processes, but we are also very interested in downstream processes. Because we know that current downstream processing is not perfect, we would like to be part of projects focusing on improvement of the processing. Also, cooperation with so many interesting partners from different countries is new for us in many ways. We have the possibility to discuss with our project partners their opinions regarding solutions to different problems. This is maybe not the biggest challenge, but something extremely valuable to us.

In the Intenso project we are focused on plasmid DNA (pDNA) production in bacterial cells. We have been working in this area for many years, from the very beginning of our company (1995). In general we concentrate on small scale production. How the project had been progressing it was necessary to provide bigger amounts of biomass. So, we did know that we will need to provide an effort to scale up. For this purpose, it was necessary to optimize the whole process. This was the challenge for us to produce enough of the material in a short time for further use in downstream processing that required tens or hundreds of grams of bacterial biomass. We provide pDNA for the testing of different downstream processing techniques, and we strive to reach the best possible results.

Universidad Nacional de Quilmes (UNQ): First findings in developing specific materials for downstream processing

Chromatographic processes are the most widely used methodology in bio-macromolecules purification on an industrial scale, however they account for more than 50% of the total manufacturing costs. The batch packed bed format is almost universally applied, which is filled with micron-scale beaded material with the appropriate adsorbent properties. In addition, in the last decade, novel adsorptive products, such as perfusive monoliths and membrane adsorbents, have found industrial applications in some bioprocessing niches. However, all these materials require an intensive manufacturing process to yield standardized adsorptive products.

Recently a novel composite fibre technology, known as gPore, has been introduced that is based on cotton fibres as adsorption material, providing high productivity and cost-effective features. Pre-swelled cellulose fibres are modified by ionizing radiation to introduce poly-methacrylates in order to add selective adsorptive properties, but preserving the original three-dimensional fibre structure. Cartridge modules filled with this fibrous adsorbent offer faster process times and are easily integrated within current industrial infrastructures. Another advantage provided by the fibrous adsorbent systems is the low-pressure drop observed during chromatography, especially at high flow rates. Moreover, adsorptive fibres can be easily arranged in an open structure that allows biomass tolerance while retaining its hydrodynamic features. This characteristic becomes an advantage, as it results in simplifying the recovery of bio products directly from a cell-containing feedstock. The described technology could add a new tool to the current downstream processing portfolio.

Aqueous Two Phase Systems (ATPS)

Downstream processing of biological products (enzymes, proteins, nucleic acids etc.) from fermentation broth or cell lysate is an important step in the production process. The greatest capacity constraints in current manufacturing platforms are no longer found in the upstream production processes, where cell culture productivity has dramatically increased over the past decade, but in the downstream purification area. The widely employed conventional techniques such as chromatography, electrophoresis and precipitation carry a considerable price while simultaneously providing low yields. They might also not be suitable for large scale production.

Aqueous two-phase systems have shown to be a valuable option for the downstream processing of several biomolecules, including antibodies (mAbs) from different complex cell culture media. ATPS were shown to be of considerable advantage in terms of process economics in the case of mAbs, especially when processing high titre cell culture supernatants, compared to the currently established protein A affinity chromatography. ATPS are formed when two water-soluble polymers (e.g. polyethylene glycol, dextran), or a polymer and a salt (e.g. potassium phosphate, sodium citrate, sodium sulfate), are mixed in aqueous solutions at a given proportion beyond the critical concentration. Separation of the proteins can be achieved by manipulating the partition coefficient of the proteins, varying the molecular weight of the polymers, the ionic strength of the salts, the relative proportion of each component, the pH etc.

ATPS can combine a high biocompatibility and selectivity with an easy and reliable scale up and capability of continuous operation. Moreover, it can overcome some of the technical drawbacks currently encountered using the established purification platforms, such as high cost, batch

operation, low productivity, scale-related packing problems, diffusional limitations and low chemical and proteolytic stability. Furthermore, ATPS allows process integration and can be used in an early stage of the bio-products purification platform to integrate clarification, concentration and purification from non-clarified cell culture media.

With regard to these advantages, ATPS have been applied in several fields such as recovery of biopharmaceuticals, environmental remediation, protein purification and extractive bioconversion.

In the frame of the Intenso Project (WP2) ATPS are applied for purification of tagged recombinant proteins, antibodies and virus-like particles expressed in different hosts (bacteria, insect cells, mammalian cells) and to integrate both up and downstream processes in order to overcome the most important bottlenecks of large-scale chromatography: cost, throughput and diffusional limitations, with the possibility of processing whole cell culture media. A purification scheme for some of these proteins was already proposed.

A large number of biotechnologically useful microbial proteins are intracellular in nature. Many enzymes/proteins produced by recombinant DNA technology are expressed intracelullarly as well. Most often, their release form the cells is performed by mechanical disintegration.

This methods suffers from many drawbacks when used on a large scale, such as the release of many contaminants – proteins, nucleic acids; loss of activity due to heat and /or free radical generation; micronization of the cell debris etc. It is largely accepted that achieving a more selective liberation of the desired product can have great impact on the efficiency and thus on the cost of the entire downstream process. Selective liberation of intracellular product, especially from cells possessing cell walls (microorganisms, plant cells) can be achieved by plasma membrane permeabilization.

As of now, the most popular method for large scale permeabilization of any type of cells is pulsed electric field (PEF) treatment.

The treatment of cells with electric pulses of an intensity in kilovolts per centimeter and durations of microseconds to milliseconds provokes changes in the membrane structure and, as a result, reversible or irreversible loss of the membrane barrier function. The generally accepted terms describing this phenomenon are "electroporation" and/or “electropermeabilization”. The irreversible plasma membrane permeabilization results in cell death, but also in the release of the soluble intracellular matter into the suspension. Pulsed electric field (PEF) treatment that induces irreversible plasma membrane permeabilization gained great popularity during recent years as an efficient method for non-thermal liquid food preservation and for extraction of biologically active compounds from microorganisms and plant cells. Over the last decade, the equipment and processes required for PEF treatment have undergone extensive development and production scale PEF systems and processes have been adopted for both commercial operations and research application.

A development of a new approach for extraction/purification of intracellular biomolecules based on a combination of continuous PEF treatment /ATPS purification seems very attractive, because it will combine a mild, selective extraction of biomolecules with mild and relatively simple and inexpensive purification/concentration.

Yeasts are among the four main expression systems (bacteria, yeast, insect cells, mammalian cells) used in the production of recombinant proteins of medical or industrial interest. In the frame of the project Intenso (WP2), the applicability of continuous PEF treatment for highly efficient and selective

liberation of recombinant proteins from different yeast species was demonstrated. Experiments on further purification (concentration) of the liberated proteins by ATPS are currently underway. Experiments on applicability of continuous PEF treatment for extraction of soluble recombinant proteins from E coli are in progress.

Intenso at the Monolith Summer School 2014 and Symposium 2014

The 6th Monolith Summer School and Symposium, 2014, was held in the beautiful coastal town of Portorož, Slovenia. With 155 participants from 26 countries it was the most successful event in its history.

The event was divided into 2 parts. First part was the Monolith Summer School (May 31st – June 1st). The knowledge gained in the lectures was applied in a series of workshops focusing on the purification of biomolecules and scale-up. During the workshops, the participants were able to see for themselves how easy it is to handle complex samples and chromatographic issues when working with monolithic supports. Workshops were intended for beginners as well as those already familiar with HPLC Chromatography of Biomolecules.

The second part of the event was the Symposium (June 1st – June 4th), which was dedicated to innovations in the field of monolith technology and production of biomolecules. 44 speakers from all over the world presented their results on several different topics:

- Theoretical aspects and characterization of monoliths
- Process design
- Protein purification
- pDNA
- Virus and pDNA purification
- Protein analytics
- Biotransformation

Additionally, young scientists had the opportunity to present their work during “Young Researchers Corner” or poster sessions, with high participation of 36 posters.

Intenso WP4 results were presented in two lectures. Petra Steppert from BOKU, Vienna, presented the progress made in the area of virus-like particle method development (»Adsorption and purification of virus-like particles by monoliths«) and Johannes Geiger from ethris presented upstream and downstream processing of mRNA (“Upstream and downstream processing of stabilized non-immunogenic messenger RNA”).

Petra Steppert compared a conventional density gradient centrifugation process for purification of VLPs to a monolith based chromatographic purification process. Both downstream processes resulted in similar product purities and yields. However, using monoliths the process time could be reduced from 19 hours to 3 hours while processing double the loading volume.

In the first part Johannes Geiger focused on the production of SNIM® RNA which starts with modular design of an mRNA production vector (specifically designed based on requirements of the therapeutic target) followed by the production of mRNA by in vitro transcription (upstream) comprising also polyadenylation and capping of mRNA molecules. In the second part the results of mRNA purification using CIM monolithic columns (BIA Separations) were presented. It was shown that CIM C4 HLD radial monolithic column under hydrophobic conditions enables mRNA purification with high yields of biologically active mRNA. Therefore CIM monolithic column based mRNA purification has a high potential to substitute currently used precipitation based mRNA purification and this way enable the production of mRNA at industrial scale in the future.