Working group: Product Isolation and Purification

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Intensified processes for selective recovery of active peptides and proteins

On June 25th, 2013, the working party on Downstream Processing of the Dutch Biotechnology Association (NBV) organized a session on “Intensified processes for selective recovery of active peptides and proteins” during the “Voorjaarsbijeenkomst” of the KNCV.
This session shows a nice collaboration between different partners (Twente University, Wageningen University, DSM, MSD and Synthon Biopharmaceuticals BV) in the development of new affinity ligands for proteins/carbohydrates, its coupling on activated matrices and use in purification processes under de vlag of Institute of Sustainable Process Technology (ISPT).

The first speaker (Guy de Roo, Synthon Biopharmaceuticals BV) gave a nice overview of the total project by efficient recovery of peptides and proteins in large quantities which remains

challenging and expensive in industrial food and biotechnology applications. Current technologies are reaching their limits with regard to throughput. The aim is to develop an efficient and cost-effective separation technology for active biomolecules from complex process streams such as fermentation broths and food waste streams. The combination of affinity chromatography in combination with membrane technology could allow selective, fast and cheap processing without pressure build-up. In this session the following three topics will be discussed.

The second speaker (Serve Kengen, Wageningen University) talked about phage display technology developing a stable ligand that can be used for industrial affinity separations. The new scaffold protein was prepared by a phage display technology by using e.g. the β-glucanase (LamA) protein from the hyperthermophile *Pyrococcus furiosus*. LamA is a thermostable protein able to bind a sugar. The protein is engineered such that it captures proteins in industrial applications. A large library of these scaffold proteins were prepared and screened for binding to target compounds. Fine tuning was performed by loop arrangements to improve the structure of the ligands. These scaffold proteins can then be covalently coupled to a certain matrix (e.g. resin beads or membranes) and used to isolate a target product from a fermentation stream. Candidate ligands were screened for binding, characterized and coupled to resin beads.

The third speaker (Willem van Berkel, Wageningen University) talked about (non)-specific coupling technologies.
The engagement of highly selective affinity proteins is a common approach to purify (bio-) molecules. Using immobilized antibodies to purify target proteins are among the first examples. However, using antibodies in affinity chromatography has several disadvantages, such as reduced chemical stability of the antibody, and high costs of preparing the affinity matrix. Based on the principle of high ligand selectivity, new affinity separation techniques are being developed, using thermostable proteins as affinity scaffolds. Hyperthermostable proteins are beneficial for many applications in biotechnology and related fields, mainly because of their thermal, chemical and structural stability. In this study, a general method is developed to immobilize a thermostable enzyme on a bead, resulting in high loading capacity and a functionally intact and stable biocatalyst. Different immobilization strategies and beads were compared, using a thermostable endo-β-1,3-glucanase as a show case.

The fourth speaker (Guy de Roo, Synthon Biopharmaceuticals BV) in the absence of Dimitrios Stamatialis (Twente University) talked about Mixed Matrix Membrane Technology (MMM) which consists of functionalized particles incorporated into a polymer support.
The project focused on: A) Particle Modification and Characterization: For the immobilization of ligands specific particles were used and the accessibility of these ligands to the target molecules (proteins, sugars) is a crucial element of this research. To achieve high accessibility and high throughput; B) Selection of polymer matrix material: Identifying the porous support material whereby different polymers were tested such as polyethersulfone (PES), cellulose acetate (CA) and polyethylene vinyl alcohol (EVAL) and the best one selected for the application; C) Mixed Matrix Material Preparation: In this task fiber membranes containing the functionalized particles dispersed in the porous support structure were prepared. The dispersion step should not influence the particle properties and the particle should not interact with the structure forming polymer but should be freely accessible. All those issues were systematically studied; D) Membrane testing in model applications: In this task the developed MMM were evaluated in model / target applications.
Overall the session attracted 25 interested persons mainly from different institutes.

Symposium Continuous Processing of Biological Products

On November 20th, 2013, the working party on Downstream Processing of the Dutch Biotechnology Association (NBV) organized a mini-symposium on continuous processing of biological products.

The first speaker, Marc Bisschops from Tarpon Biosystems, gave an overview of how various companies in the biopharmaceutical industries, are exploring options to transform their batch manufacturing into continuous platforms. The presentation covered three case studies and addressed some of the technological and regulatory challenges associated with continuous processing of biopharmaceutical products.

Marco Giuseppin, CTO at AVEBE, presented how a continuous protein recovery plant was integrated into an existing potato starch manufacturing factory. The protein recovery included an innovative combination of multicolumn chromatography (or SMB technology) and expanded bed adsorption. Giuseppin addressed some of the challenges associated with integrating such continuous manufacturing concepts in an existing plant that uses highly variable feed stocks, such as potatoes.

The last speaker at the minisymposium was Reiner Graub from Pall Corporation. This presentation covered the successful implementation of continuous production technologies in the last steps of the beer processing. The presentation clearly demonstrated the impact of continuous processing on footprint and process economy.

Figure 1: *Speaker Marco Giuseppin presents the continuous protein recovery system implemented at AVEBE in Gasselternijveen.*

The mini-symposium was attended by approximately 35 people, mainly from the industry.

Activities for 2014

1) One or two sessions at the 15th NBV conference, Reehorst, Ede (27-28 May)
2) Seminar on vaccins together with the bioprocessing working group in October/November 2014

Goal of the Working group on Downstream Processing

The goal of the working group Downstream Processing (Product isolatie en Zuivering) is to keep the members up to date with novel (inter) national activities in the area of separation technologies and exchange information with each other. This can be reached by keeping the website up to date with actual information, symposia, courses, etc. Overall the coordinators of this working group are always open to new suggestions to improve the goals of the working group Downstream Processing.