CONTINUOUS PROTEIN PRECIPITATION – A ROBUST ANTIBODY PURIFICATION METHOD WITHOUT THE NEED FOR STEADY STATE CONDITIONS DURING CONTINUOUS INTEGRATED PRODUCTION.

Daniel Burgstaller, University of Natural Resources and Life Sciences, Vienna, Austria
daniel.burgstaller@boku.ac.at
Peter Satzer, University of Natural Resources and Life Sciences, Vienna, Austria
Steffen Großhans, Karlsruhe Institute of Technology (KIT), Karlsruhe, Germany
Simon Caserman, National Institute of Chemistry Slovenia, Ljubljana, Slovenia
Alois Jungbauer, University of Natural Resources and Life Sciences, Vienna, Austria

Key Words: Continuous, integrated, precipitation, steady-state, antibody.

Antibody precipitation as capture step is an alternative technology to chromatography, because it is a fully continuous method. Bioreactor effluent from a perfusion reactor can be directly processed without surge tanks and the method can be combined with flocculation. We hypothesized that a continuous precipitation and flocculation reactor can be operated under non-steady state conditions. This will allow the design of reactors with extremely short residence time. In batch precipitation steady stats is achieved when the floc size does not change over time. We developed a continuous capture step for antibodies by protein precipitation with PEG 6000. Precipitation and re-solubilization conditions were established by a high throughput approach in batch mode. Continuous precipitation was realized by a tubular reactor design containing static mixers. The particle size distribution of the product precipitate was on-line monitored by Focused Beam Reflectance Measurement (FBRM) in a flow cell. Various hold times (2 to 20 minutes) of the cell culture harvest and different product concentrations were used to simulate changes during continuous fermentation. Precipitate was collected and separation was performed by centrifugation. High molecular weight impurities were reduced by a factor of 5 to 10, yields of 90% and purity increase by a factor of 2.5 were achieved according to size exclusion chromatography. Using FBRM we were able to demonstrate that different residence times during precipitation do not significantly change the particle size distribution. Stable performance concerning product quality (yield, purity and high molecular weight impurities) for different residence times were demonstrated for up to 90 minutes runtime. The influence of product concentration on the precipitate was quantified by FBRM. We demonstrated that protein precipitation is a robust and feasible capture step for mAb purification that does not require steady state conditions and that such continuous reactors are not compulsory operated at steady state conditions. Optimization potential was identified and can be realized during upscale, and higher yields and better performance can be reasonably expected for larger scales.