

TRULY CONTINUOUS DOWNSTREAM PROCESSING OF ANTIBODIES, OVERCOMING BOTTLENECKS

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Continuous precipitation of monoclonal antibodies is a low-cost alternative capture step to the state-of-the-art protein A affinity chromatography. Similar purity levels can be achieved by the combination of orthogonal precipitation methods such as CaCl₂ and PEG precipitation. First, DNA and high molecular mass impurities are precipitated by addition of CaCl₂. Depth filtration is used for the removal of such insoluble matter. Antibodies are then captured from this pre-treated culture supernatant by means of PEG precipitation. One way to reduce compaction of the precipitate during harvest is membrane filtration instead of centrifugation. A loose precipitate allows faster redissolution and increases the yield. Continuous membrane filtration can be realized by running at least two staggered filtration processes in parallel. However, this strategy interrupts the fully continuous operation and the antibody must be harvested in cycles. Continuous multi-stages microfiltration with feed-and-bleed configuration generates instead a fully continuous mass flow and the precipitate can be concentrated, washed and conditioned for the subsequent polishing steps.

The integration of unit operations in our system is accomplished in a pool-less fashion, thus reducing the plant footprint and expenses for investments in surge tanks. Furthermore, the RTD becomes narrower and start-up and shut-down operation faster. This integrated set-up generates a truly continuous mass flow of product by the adoption of tandemized equipment to avoid any flow interruption.

The system was tested by using clarified cell culture supernatant from a perfusion process. After pre-treatment with CaCl₂, the product stream was depth filtered in continuous and the antibodies in the clarified feed were continuously captured by in-line addition of PEG at the inlet of a tubular reactor. Continuous tangential flow filtration was performed for the concentration and washing steps. After the ramp-up, the precipitated product was harvested with a continuous and constant mass flow for several days. The purity, yield, and quality of the antibodies was comparable to batch-wise operation. This system is implemented in an automated skid for integrated continuous manufacturing of antibodies at pilot scale.