PILOT-SCALE INTEGRATED CONTINUOUS BIOMANUFACTURING FOR MONOCLONAL ANTIBODIES INCLUDING MILD pH

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We have developed and scaled to pilot-scale an integrated continuous bioprocess (ICB) suitable for the production of pH-sensitive antibodies. The low pH in the elution of the protein A capture step can cause antibody aggregation, generating both a yield reduction and a higher risk for the final substance safety.
A process, using CHO-K1 cells producing monoclonal antibody (mAb), was developed at small scale in 200 mL stirred tank bioreactor in steady-state perfusion integrated to a continuous downstream process (DSP). The cells were expanded in a seed bioreactor in perfusion mode until a density of 70 x 1E6 cells/mL was reached. In the production bioreactor, after expansion, the cell density was stably maintained at 100 x 1E6 cells/mL in steady-state with a perfusion rate of 1.5 reactor volume /day. The previously reported strategy of targeted feeding of glucose, TAFE [1], was used to determine the fed medium, based on commercial media and feed concentrates. In the continuous DSP, a new protein A resin, Z_{Ca}, was used. The Z_{Ca} resin, based on a calcium-dependent interaction, had an elution at mild pH 5. The capture step operated with a three-column PCC was followed by a solvent-detergent step for virus inactivation, a CEX step in bind/elute mode, and an AEX step in flow-through mode (ÄKTA pure) [2]. The DSP had been optimized in small scale before its integration with the perfusion culture [3].

The ICB was then scaled up in a XCellerex-XDR-50 bioreactor at 30 L working volume, using the same ÄKTA equipment for the DSP as in small scale. The culture, integrated with continuous DSP, was stably maintained at 100 x 1E6 cells/mL at high viability during 17 days. Antibody aggregation was barely detected (SEC analysis) after the Z_{Ca} protein A capture operated at mild pH, showing that this ligand is highly suitable for the purification of pH-sensitive antibodies. The control system Orbit ensured the USP-DSP integration and automated DSP in both scales [4]. The glycosylation was stable at steady state and the levels of host cell proteins and DNA impurities were very low in the final substance. This ICB process at pilot scale had an overall DSP yield of ≈ 90%, which can be attributed to the absence of aggregation thanks to the mild pH capture and to the optimized integrated process, and resulted in 1 g purified antibody/L/day.

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