TOOLBOX FOR EFFICIENT DEVELOPMENT OF HIGH CELL DENSITY PERFUSION PROCESS, INTEGRATED WITH CONTINUOUS DSP

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We have successfully developed and realized at pilot scale (50 L XCellerex-XDR-50 bioreactor) a high cell density perfusion process (HCDP) of CHO-K1 cells for the production of a monoclonal antibody (mAb), stabilized at 100 x 1E6 cells/mL steady state, and integrated to a continuous purification process. The purification process included a capture step using a novel calcium-based protein A affinity ligand, Z_{Ca}, with elution at mild pH 5, and operated with a three-column PCC. This step was followed by a solvent-detergent step for virus inactivation. Finally, the polishing consisted in cation exchange and anion exchange steps [1] [2]. Advantageously, the same ÄKTA equipment was used at small and pilot scales. Thanks to the very gentle pH conditions, aggregation of mAb did not occur during the purification process. This, together with the process automation and optimization, resulted in a yield of the integrated continuous DSP at pilot scale of 90%, with very low final impurity levels of HCP and DNA.

The success of this realization in pilot scale stems from very good knowledge of the perfusion and the purification processes. Here we present our strategy for medium and process optimization of HCDP. The design of the perfusion process is based on targeted feeding of the sugars and the amino acids providing the desired cell metabolism, very efficient usage of these substrates and minimized by-product formation, i.e. lactate, ammonia. This strategy can be used e.g. for glycosylation tuning, for extremely low cell specific perfusion rate down to 15 pL/cell/day or for translation of fed-batch into perfusion process [3] [4]. The experimental systems are 200 mL stirred tank bioreactors and microbioreactors ERBI of 2 mL working volume. Here we compare and exemplify these in medium design by simplex mixture Design of Experiment (DoE) with optimization objective to maximize the total volumetric productivity of mAb, by maximizing the mAb cell specific productivity and minimize the cell growth to reduce the loss associated to the discarded cell bleed at steady state. The media formulations are tested in the perfusion microbioreactors Erbi, which demonstrated cell growth rate, production of mAb and by-products, as well as mAb N-glycosylation similar to stirred tank bioreactors with ATF. The selected formulation is finally validated in stirred-tank bioreactor.

Funding: VINNOVA [grant 2016-05181]. The authors thank as well Erbi Breez, USA.

References

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