STRAIGHT-THROUGH PROCESS DEVELOPMENT OF UP AND DOWNSTREAM INTEGRATION OF MONOCLONAL ANTIBODIES PRODUCTION USING FLOCCULATION, AEX AND ONE PASS TFF

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The monoclonal antibody (mAb) market has been presenting a significant growth rate in the last two decades, which increased the interest of biopharmaceutical companies in this product class. Many improvements have been achieved in the upstream processing of mAbs, leading to significant increases in bioreactor titers. However, the production costs are still high, especially due to downstream processing costs, which can represent a major part of the of overall production costs. Traditional mAb platform processes include a very selective, but high-cost Protein A (PrA) affinity chromatography as the first purification step (capture). Several approaches have been recently explored in order to replace PrA chromatography. In this work, we propose a new, low-cost strategy for integrating clarification and capture step for mAbs using flocculation followed by a straight-through process with single-pass tangential flow filtration (TFF) and suspension anion-exchange (AEX) chromatography. First, the recombinant anti-IL8 mAb were produced by CHO-DP12 cells (ATCC, USA) in shake flasks at 180 rpm and 37°C using TC-LECC medium (Xell, Germany). After harvest, cells were flocculated using 5 pg per total cells at pH 6.5, allowing 15 min for settling of cells. Subsequently, the resulting supernatant and a Q-Sepharose resin (GE, Sweden) were pumped in equal amounts to a vessel, where a residence time for AEX adsorption of 15 min was applied, with the aim of allowing contaminants to adsorb to the resin. The resulting supernatant/AEX resin suspension was pumped out of the vessel into a 0.22-µm hollow fiber system (GE, USA). The mAb was recovered in the permeate, whereas the AEX resin remained in the retentate and could undergo elution, regeneration and sanitization for reusing. Two process variations were evaluated (Table 1), which were combined resulted in 6 different process strategies: (i) the ratio of clarified supernatant to AEX resin; (ii) the use of a device for cell/flocs retention named inclined lamella settler (Biotechnology Solutions, USA) and depth filter Clarisolve (Merck, USA), both were placed after the flocculation step to ensure a cleaner supernatant and to allow reducing the cell settling time. The integrated clarification-capture process showed to be simple and fast. Steady-state conditions were obtained during adsorption and filtration for all conditions studied. The average recovery of mAb during the steady-state was 48.5% ± 2%, which means a loss of approximately 3% of mAb product, since it was 2-fold diluted by the 1:1 mix with the resin suspension. However, considering the overall process, from start to final permeate recovery, global yields between 61% and 90% were obtained. These results are mainly related to the void volume of inclined lamella settler. The best global recovery (90.4%) was obtained when the depth filter was included in the process. Regarding impurities removal, in all 6 process strategies evaluated more than 85% of DNA was removed, and approximately 70% of HCP removal could be achieved when depth filter was used. Taking into account that two different supernatant/AEX resin ratios tested, a lower supernatant/resin ratio (41) provided a higher DNA clearance (86 fold), compared to less than one third of this clearance when sample/resin ratio was doubled to 82.

Table 1: Straight-through process variations studied.

Process	Viable cells x10^6/mL	Cell viability (%)	Retention device	Ratio Sample/AEX
1	7.3	87.6	None	82
2	11.9	95.2	Settler	82
3	15.1	88.8	Settler	82
4	19.3	80.4	Settler	41
5	12,1	91.9	Filter	41
6	8.3	87.7	Filter	41