Introductions

The cell culture medium is critical for a cost-effective and efficient perfusion process. A common strategy for perfusion process control is to maintain a constant CSPR. With this approach, the volume of perfused medium per cell is kept constant during the entire process, meaning that the CSPR is determined by limiting nutrients in the culture medium. Using an unbalanced medium, single nutrient components will be limiting, whereas nutrients that are available in excess will be flushed out together with the retentate. The CSPR will be high and the medium will not be fully utilized. Hence, the more tailored the medium composition is to meet the needs of the specific clone being used, the better the medium will be utilized and the CSPR can be lowered. A balanced perfusion medium is required to achieve high viable cell densities (VCDs) in a process run at low CSPR (Fig. 1).

In this study, we present two approaches for the development of a high-performing perfusion medium from an existing medium platform. Both methodologies are easily applicable for other cell culture media and have great potential to meet many of tomorrow’s demands within biopharmaceutical manufacturing when combined with a continuous downstream operation. However, the steady-state approach showed improved performance compared with the batch approach with regard to cell growth and productivity. Although taking twice as long to develop, the steady-state approach resulted in a final process with more than a 70% decrease in cell-specific perfusion rate (10 pL/c/d) compared with the starting process conditions (90 pL/c/d) and a volumetric productivity of approximately 0.5 g/L/d. The achieved reduction in CSPR has great impact on manufacturability and process economics.

Results

Steady-state approach

Spent medium analysis

The cell-specific amino acid consumption rates were measured in perfusion cultures at varying CSPR. The CSPR was stepwise decreased and samples for amino acid analysis (AAA) were taken at the end of each steady state condition as indicated by the arrows in Figure 5.

Heat map

Based on the results from the spent medium analysis, a heat map of the seven limiting amino acids at different CSPR is shown in Figure 6. Some amino acids become limiting first at low CSPRs, whereas other amino acids are limiting already at high CSPR. Best performance in this study was achieved when ActiCHO P medium was supplemented with 7% ActiCHO Feed A and 1% ActiCHO Feed B.

Verification in perfusion cultures

Performance of optimal prototype medium design from each approach was evaluated in perfusion cultures by running the cultures to maximum cell density, using a constant volumetric perfusion rate of 1 RV/d without bleed. The results are shown in Figures 7 and 8. With the batch approach, a window of opportunity was identified around 40 MCV/mL, CSPR ≈ 25 pL/c/d, whereas for the steady state approach, a window of opportunity was identified around 50 MCV/mL, CSPR ≈ 20 pL/c/d. These conditions, under which the cell-specific productivity was stable and the growth rate was low, were verified in steady-state perfusion runs (Fig. 9). The cell density was maintained stable by daily bleed.

Figures

Fig. 1. Impact of CSPR on the volumetric perfusion rate (RV/d).

Fig. 2. Viable cell concentration for the cultures included in the DoE screening study.

Fig. 3. 4D contour plot for ActiCHO P medium, Feed A, and Feed B concentrations. Final VCDs are shown in the upper panel and have an R² value of 0.87 and a Q² value of 0.64. Final titters are shown in the lower panel and have an R² value of 0.92 and a Q² value of 0.78.

Fig. 4. Verification of the best prototype medium developed using a batch approach. (A) Viability, cell concentration (Cv), and CSPR in the perfusion run at 1 RV/d. Run performed to determine maximal VCD for this study. (B) IgG titer, viable cell concentration (Cv), product-specific IgG, and cell-specific growth rate (µ) in the perfusion run at 1 RV/d. Run performed to determine maximal VCD for this study.

Fig. 5. Sampling for analysis of amino acid consumption rates at the end of each steady state condition.

Fig. 6. Heat map of limiting amino acids at different CSPR. Yellow = between 20% and 30% of initial ActiCHO P medium. Red = below 10% of initial ActiCHO P medium.

Fig. 7. Verification of the best prototype medium developed using a steady-state approach. (A) Viability, cell concentration (Cv), and CSPR in the perfusion run at 1 RV/d. Run performed to determine maximal VCD for this study. (B) IgG titer, viable cell concentration (Cv), productivity (qP), and cell-specific growth rate (µ) in the perfusion run at 1 RV/d. Run performed to determine maximal VCD for this study.

Fig. 8. Verification of the best prototype medium developed using a steady-state approach. (A) Viability, cell concentration (Cv), and CSPR in the perfusion run at 1 RV/d. Run performed to determine maximal VCD for this study. (B) IgG titer, viable cell concentration (Cv); product-specific IgG, and cell-specific growth rate (µ) in the perfusion run at 1 RV/d. Run performed to determine maximal VCD for this study.

Fig. 9. Perfusion culture runs at steady-state conditions. (A) Confirmation of conditions identified using the batch approach. (B) Confirmation of conditions identified using the steady-state approach.

Conclusions

We present two separate approaches for developing a high-performing perfusion medium from an existing medium platform. Both methodologies are easily applicable for other cell culture media and have great potential to meet many of tomorrow’s demands within biopharmaceutical manufacturing when combined with a continuous downstream operation. However, the steady-state approach showed improved performance compared with the batch approach with regard to cell growth and productivity. Although taking twice as long to develop, the steady-state approach resulted in a final process with more than a 70% decrease in cell-specific perfusion rate (10 pL/c/d) compared with the starting process conditions (90 pL/c/d) and a volumetric productivity of approximately 0.5 g/L/d. The achieved reduction in CSPR has great impact on manufacturability and process economics.