Experimental design and small-scale model for high-performing perfusion media and processes scalable to 50 L bioreactors

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Perfusion media development for scalable processes

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Abstract

Cell culture perfusion processes are considered optimal for a truly integrated continuous biomanufacturing pipeline. The nutrient-rich but balanced media should be designed to support very low cell-specific perfusion rates (CSPR) that minimize media consumption while maximizing viable cell yields and productivities. Optimized processes at low CSPR drastically reduce equipment costs, lab space, and product dilution. Finally, operating at very low CSPR will allow for mammalian cell bioprocesses to run as true chemostat cultures in the future. In this study, we demonstrate a general workflow to develop high-performing perfusion media using small-scale models and transfer the process to a 50 L-scale at CSPR of 20 μL/cell/day.

Materials and methods

Cell line: HyClone™ Chinese hamster ovary (CHO) cells (CHO-K1, IgG1)

Basal media: HyClone CDM4NS0 or Hyclone ActiPro™

Feed supplements: see Figure 1

Analytical methods: Vi-CELL™ (viable cell density, VCD), BioProfile™ 100 plus (glucose, lactate, glutamine, glutamate, NH4+), Osmomat 030 (mOsm/kg), Octet™ QK Siter

Design of Experiment (DoE, Steps 1 and 2)

Three DoE levels (-1, 0, +1):
- Level -1: 10% or 20% of 3D media
- Level 0: half-maximum spike concentrations
- Level +1: all Cell Boost™ supplements were mixed according to total amino acid and glucose spiked into basal media to reach 400 mOsm/kg; design of DoE matrix and establishment of final statistical models were performed using MODDE™ software

Semi-continuous small-scale perfusion models (Steps 2 and 3)

Start VCD: 10 to 100 cells/mL in 10 ml spiked basal medium

One volume exchange per day (1 reactor volume (RV)/8) by centrifugation at 100 g/7 min after bleeding if applicable

220 rpm at 50 mm shaking diameter in a Kuhner shaker instrument at 7% CO2, and 85% humidity at 37°C

Biorеactor verification runs (Step 4)

Optimized CDM4NS0 or ActiPro perfusion media was applied to perfusion bioreactors using a ReadyToProcess WAVE™ 25 or Xcellerex™ XDR 50 bioreactor; cells seeded at 1 × 10⁶ cells/mL, in unspiked basal medium, and perfusion initiated on days 2 to 4 at a working volume of 500 ml or 40 L, culture parameters set to control > 30% dissolved oxygen (DO), 37°C, pH 6.8 to 7.0

Results

A generally applicable perfusion medium development workflow was applied to different HyClone CHO basal media: ActiPro and CDM4NS0. In a first screening round (Fig 2, step 1) beneficial effects of Cell Boost supplements 1, 5, 7a, and 7b were identified using a DoE approach in spiked batch cultures. The pre-selected supplements were subsequently applied to a second DoE using 10 μL shaking cultures in a semi-continuous perfusion mode by daily media exchange (Fig 2, step 2), where the primary objective was to fine-tune the ratio of pre-selected Cell Boost supplements. High VCDs of more than 50 × 10⁶ cells/mL in a quasi steady-state were reached. Spiking basal medium with Cell Boost supplements improved viabilities and daily titers, with values up to 1 g/L. Subsequent bleeding experiments in semi-perfusion cultures (Fig 2, step 3) revealed higher maintained growth rates at higher bleeding rates, correlating with higher specific productivities. Despite lower steady-state VCDs, increased specific productivities resulted in the titers increasing by 20% when a 30% daily bleed was used. Ni-glycosylation profiles of antibodies produced in the semi-continuous models showed a decreased galactosylation patterns at later process times.

Two novel perfusion media developed within this project and based on basal CDM4NS0 or ActiPro and Cell Boost 1 and Cell Boost 3 were applied to different bioreactor perfusion verification runs. Using a continuous volumetric perfusion rate, the minimum CSPR of 10 μL/cell/day was determined to generate the highest VCD of more than 200 × 30° cells/mL. A similar high VCD was reached with ActiPro + Cell Boost 1/3 by using a constant CSPR of 15 to 30 μL/cell/day to reduce medium consumption. The novel perfusion media were also applied to bioreactor production runs at a constant VCD of 50 × 30° cells/mL at a 500 ml or 40 L-scale. An increase of galactosylated glycan species was observed over process time, and a good correlation of various bioreactor parameters compared to the 30% bled small-scale model was identified. Major differences were only found for the glutamate/glutamine/NH4+ behavior, which might be responsible for the discrepancy of the terminal galactosylation profile.

Conclusion

- A DoE-based workflow was developed to leverage established feed supplements for definition of novel, high-performing perfusion media.
- Small-scale models in semi-continuous perfusion mode were used to screen different conditions within a single operator.
- A minimum CSPR of 10 μL/cell/day was determined by constant volumetric perfusion rates in a ReadyToProcess WAVE 25 bioreactor to reach 200 × 30° cells/mL.
- A steady-state production perfusion run was scaled up to an Xcellerex XDR 50 L bioreactor.
- Glycosylation increased in galactosylated species in bioreactor perfusion runs but decreased in the semi-continuous models, likely due to higher amounts of ammonia accumulation.
- Critical culture parameters were very similar between bled small-scale cultures and the perfusion bioreactor at similar CSPRs.