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Perfusion medium considerations

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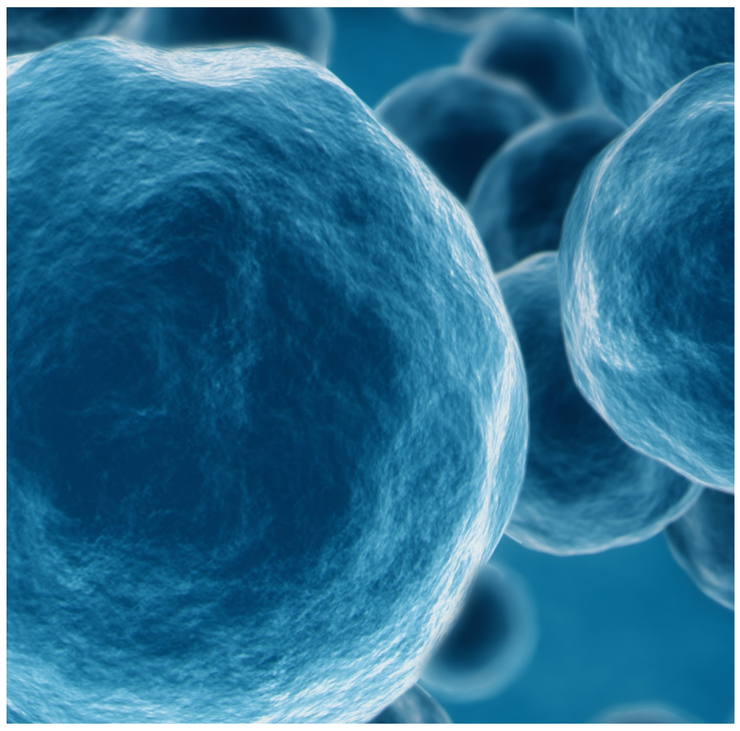

How to choose a perfusion medium

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There are many factors to consider when choosing a perfusion medium. Two such factors are ease of use and cost-effective performance. Related to ease of use, consider what is a reasonable shelf life and what the reconstituted shelf life should be to provide you with flexibility when preparing and storing the medium. Additionally, and especially when it comes to the larger volumes of medium generally needed for perfusion work, it is important for the medium format to be easy to work with. The Gibco™ Advanced Granulation Technology™ (AGT™) format supports fast and simple reconstitution. This format saves time and reduces the number and complexity of steps, and common errors when reconstituting the medium. No matter the format, the formulation should be functional as both a seed train medium and a production medium capable of supporting high cell densities and productivity at lower medium exchange rates, ideally as low as 1 vessel volume per day (VVD). Lastly, the medium should support cost-effective scale-up.

What to look for in a catalog perfusion medium

- **Easier validation:** Using an animal origin–free, protein-free, and chemically defined medium facilitates validation and helps with consistency across multiple runs, keeping you on a fast timeline to market.
- **Flexible cell line use:** A medium that is deficient in L-glutamine can be used in glutamine synthetase (GS)-based systems. A medium deficient in hypoxanthine and thymidine is compatible with dihydrofolate reductase (DHFR)-based systems. And a medium that is protein-free allows for additional growth factors, peptones, or hydrolysates to be added to or omitted from the medium according to cell line and process needs.
- **Supports dynamic process needs:** The medium needs for adaptation and typical seed train work are considerably different from those of high–cell density perfusion production. A “high medium depth” or “rich” medium (high concentrations of nutrients) is important for supporting high viable cell densities (VCD) and productivity at lower medium exchange rates (lower VVD). However, some cell lines struggle in “rich” medium or may have a slower growth rate when operating at lower VCD during adaptation, passaging, and seed train work. In some cases, multiple medium formulations may be needed to fully optimize a process workflow. However, a formulation that performs well concentrated or diluted can help support the process workflow, enabling increased productivity while reducing the need to stock multiple formulations.
- **Cost effectiveness:** Look for medium formulations that are able to maintain a high cell density and productivity at low operating VVD medium exchange rates and reasonable volume pricing to keep the cost of goods (COG) manageable.
- **Supports your process and scale-up:** Make sure there is assurance of supply and that you have confidence in the manufacturer’s ability to stock and produce media to meet your demand. Look for multiple redundant production facilities and global warehousing. Does the manufacturer provide a service to help optimize and tailor a formulation to your process and cell line to hit needed quality targets?
- **Shelf life and format:** For the larger quantities of medium generally needed, a liquid format is impractical. Dry powdered medium (DPM) and AGT formats should have shelf lives of more than 12 months and minimize storage space.

Case study: N-1 perfusion for accelerating the seed train

Perfusion can be used to accelerate the seed train, skip vessel stages, or achieve high seeding density in a production reactor. In this case, a mid-process switch in perfusion medium was performed, which required direct adaptation of two different clones that were initially frozen, thawed, and grown in a chemically defined fed-batch formulation at bench scale. An N-1 perfusion seed train was performed with Gibco™ High-Intensity Perfusion (HIP) CHO Medium. Both clones transitioned well to the medium mid-process and were able to reach the desired cell density a full day sooner than expected and with higher final cell density than expected (Figure 1). The medium exchange rate was 1 VVD, and tangential filtration flow (TFF) was used for cell retention. It should be noted that switching the medium mid-process should always first be verified as acceptable.

The goal of N-1 is to reach a target number of viable cells as fast as possible. So, it is critical that the medium used maintain a high growth rate both at low and higher VCD. A cell line sensitive to concentrated media may need to start out in diluted medium and can be switched to higher concentrations as VCD increases to maintain a high growth rate at moderate to low maximum VVD. This can generally be done in two ways: by increasing the concentration in stages once at target maximum VVD, or by seeding at a lower concentration and perfusing a higher-concentration medium starting at a very low VVD and scaling up with the cell mass being supported over time.

Implementing N-1 perfusion will allow for quicker and higher-density seeding of a production reactor, making for a more aggressive overall process.

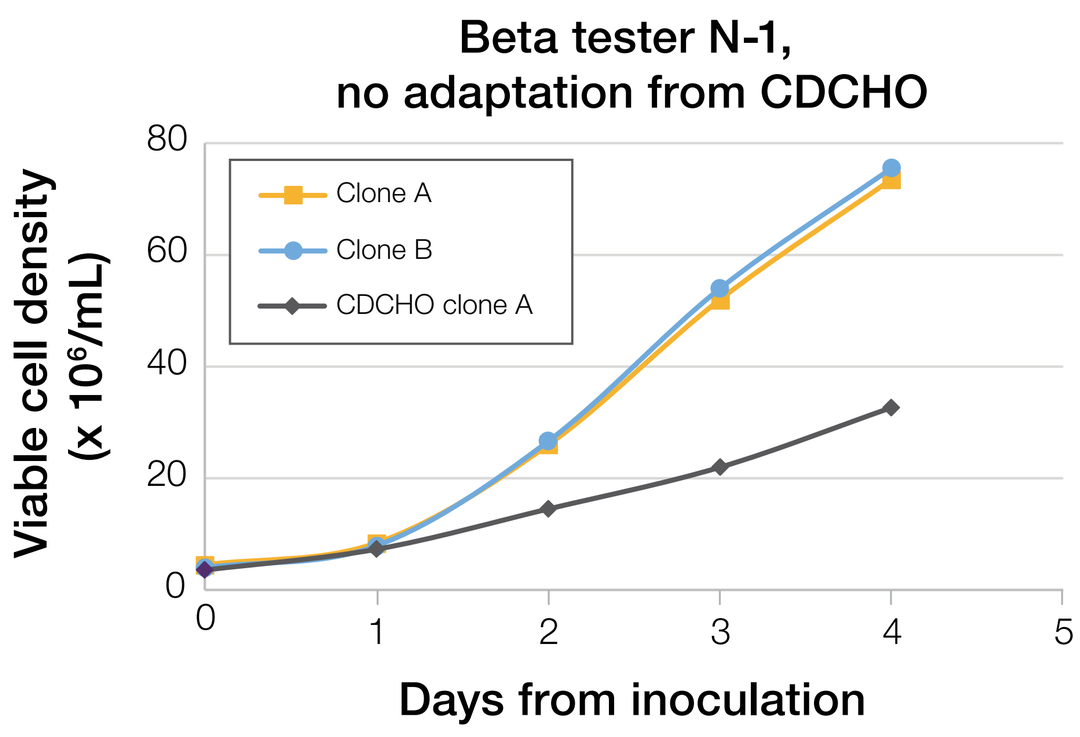

Figure 1. N-1 perfusion seed train VCD (x 10^6/mL) of two clones with a control condition using a conventional batch seed train operation.

Case study: Concentrated fed-batch for increasing productivity

Batch-like perfusion operation does not target a steady state and is commonly 14 to 20 days in duration. Due to the short duration it is important to reach high VCD quickly and produce rapidly. Your medium should support an early high growth rate but also provide enough nutrient density for the cells to maintain production through the run, or it will risk triggering a sharp crash. In this example, a concentrated fed-batch perfusion process was explored to improve the performance of a lower-producing cell line at the Bielefeld University of Applied Sciences (Bielefeld, Germany). The following conditions were used:

- HIP CHO Medium at 1.2 VVD and TFF to retain the cells and product in the reactor
- Fed-batch medium and 2X Gibco™ EfficientFeed™ C+ supplement
- A simple-batch (glucose-only) operation using the original process medium as a control

During the concentrated fed-batch perfusion run, the cell line achieved greater than 120 million viable cells/mL, compared to a peak of 20–25 million viable cells/mL in the simple-batch and fed-batch conditions (Figure 2).

The fed-batch condition displayed a large increase in productivity compared to the simple-batch condition, achieving a >5-fold increase in titer.

Additionally, HIP CHO Medium further intensified the productivity over the fed-batch condition nearly 2-fold. When combined with the notably increased cell density, this resulted in a >41-fold increase in titer. Further, at this product concentration, a downstream concentrating step was no longer required.

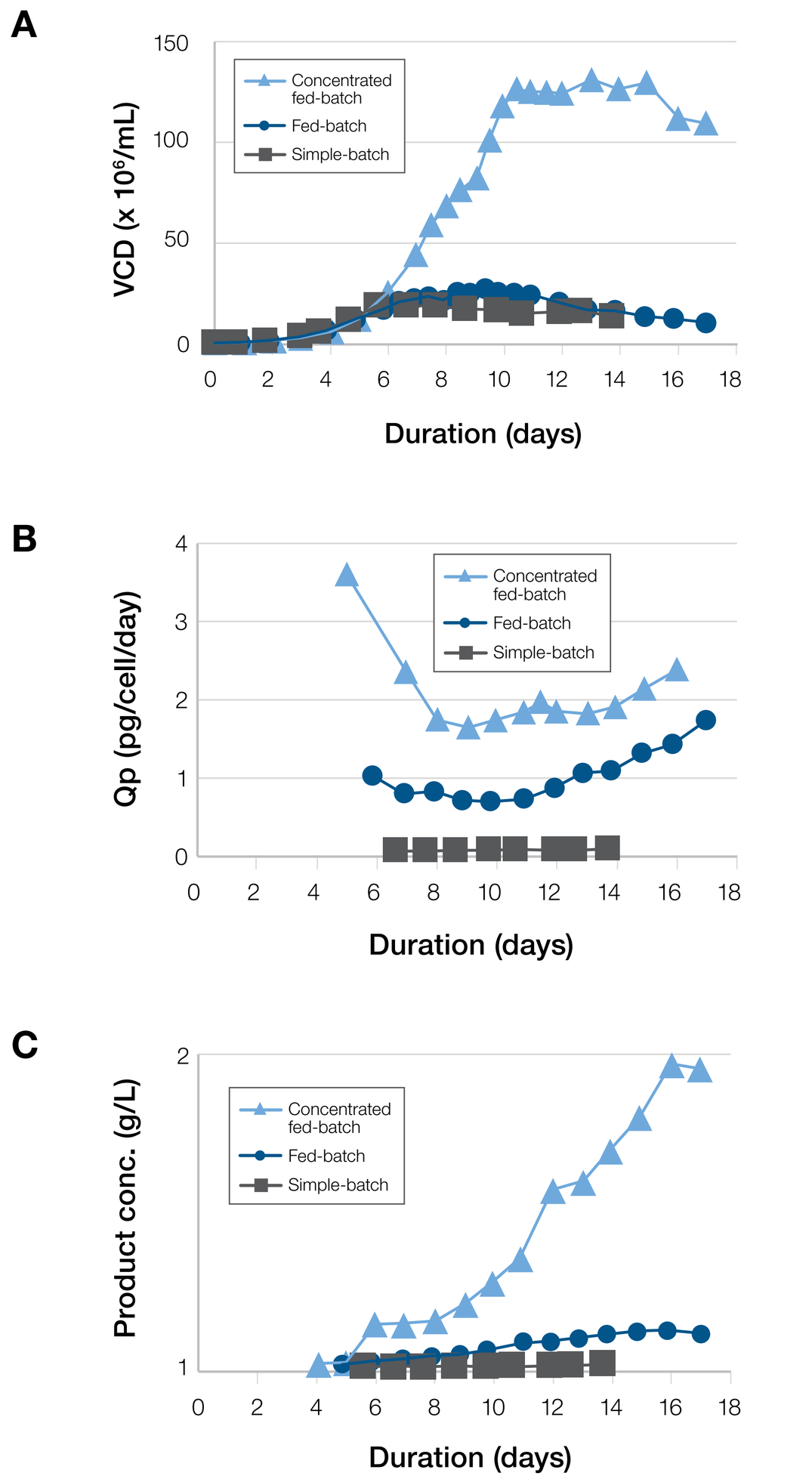

Figure 2. Concentrated fed-batch compared side by side with fed-batch and simple-batch (glucose feed only) operation. (A) VCD, **(B)** productivity (Qp), and **(C)** cumulative product concentration.

Because a “batch-like” perfusion operation happens in a shorter length of time (commonly 14 to 20 days), it is important to reach high VCD quickly while avoiding a sharp crash immediately following peak VCD. Achieving a balance of high initial log growth rate, peaking “gently”, and maintaining productivity are important. Your medium should support a higher concentration, but also allow targeted restrictions to prevent an excessively high peak VCD, which will risk triggering a sharp crash. In this case glucose was restricted at peak VCD to limit further cell growth while maintaining high production and preventing a crash in maintained cells. This approach allowed a high growth rate with a controlled VCD limit to prevent the cell mass from depleting all nutrients.

Case study: Continuous perfusion at varying medium concentrations

Most commonly associated with perfusion is a continuous perfusion process. Here the goals are to quickly achieve and maintain a process steady state with a specific quality profile for a period of time, usually limited by cell line stability or cell retention fouling risk. This case study shows steady-state perfusion operation of the same cell line at two concentrations of medium.

A cell line was thawed, passaged, and expanded in 0.66X HIP CHO Medium (faster growth rates were observed with this clone at low cell density). It was seeded into two 3 L stirred-tank vessels at 2 L operating fill volume, again at 0.66X concentration. The continuous perfusion run was started on day 3 with ATF2 filters at 1 VVD. The bleed was adjusted to actively target 95% cell viability to mimic a process with very high product-quality needs. One vessel (in blue in Figures 3–5) continued using 0.66X medium. The other vessel (in red in Figures 3–5) was perfused using 0.66X

For continuous perfusion, the primary goal of a medium is to sustain the culture and production at a steady state. A common misconception is that the cell growth rate must be reduced to limit bleed. Cell bleed ultimately is used to maintain a target quality profile, which generally correlates to maintaining a percent cell viability. So the true goal of a continuous perfusion medium is to minimize the cell death rate of a sustained cell line. This allows continuous operation at a higher VCD and lower percent bleed. Additionally, it is critical that a continuous perfusion medium sustain productivity over time. This means that steady-state metabolites can continue to facilitated production, and the formulation has no components that can build up in cells and risk productivity loss over time.

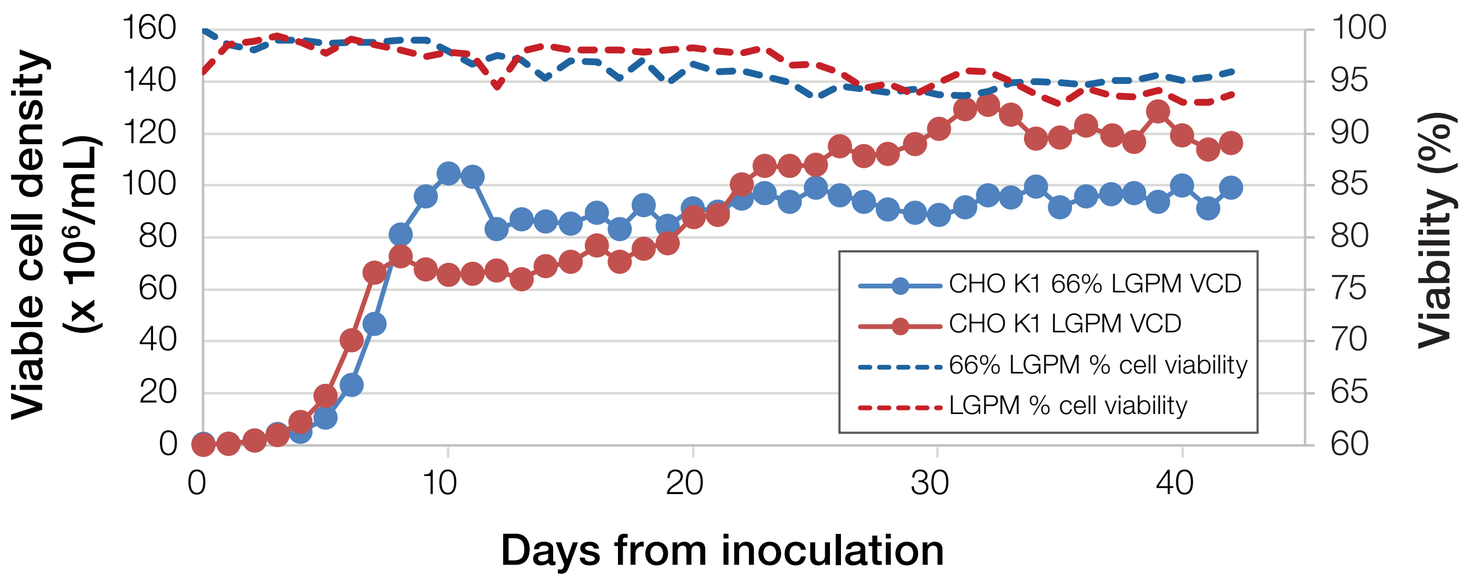

Figure 3. Viable cell density and percent viability of continuous perfusion operating with two different concentrations of the HIP CHO Medium. Fully concentrated medium was initially excessively bled causing an excessively high percent viability and lower-than-necessary VCD.

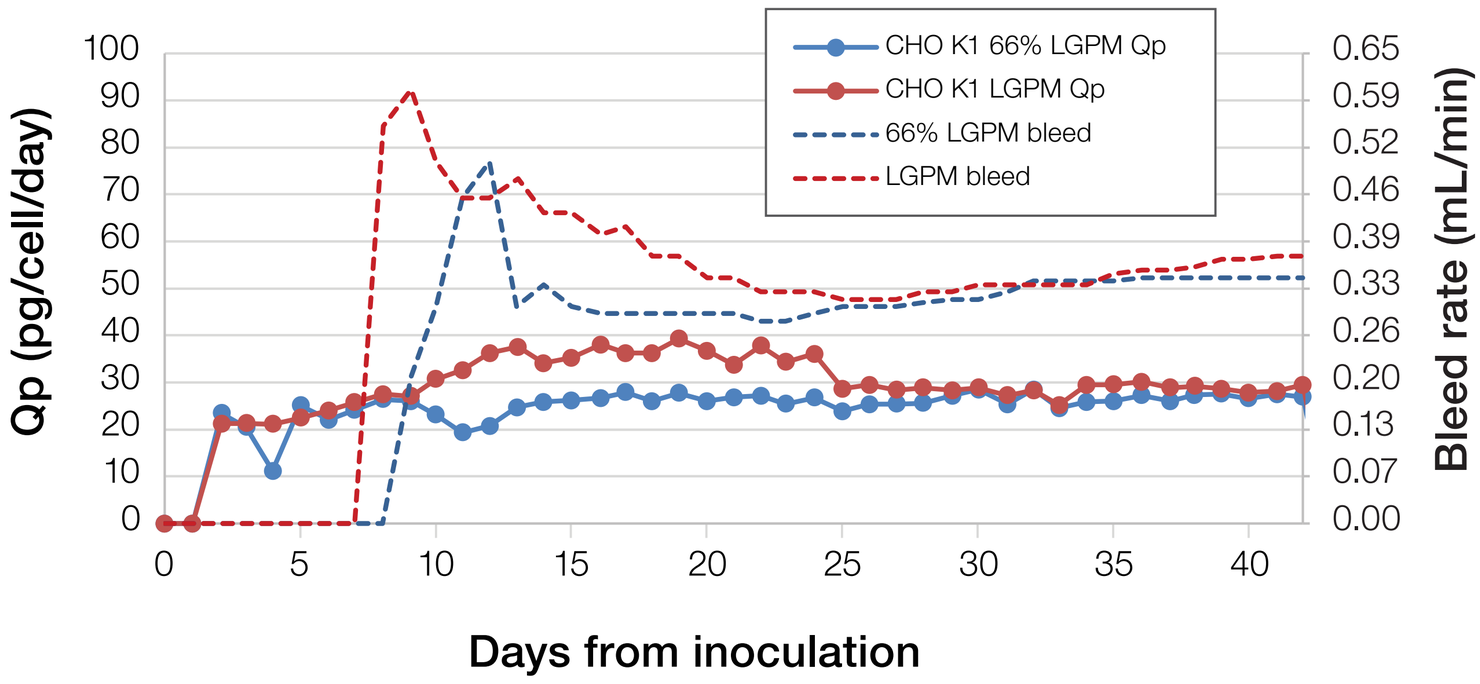

Figure 4. Bleed rates of the two continuous perfusion vessels, and volumetric productivity (Qp). Initial bleed is high to prevent excess cell density and prevent percent viability from falling below the target. Note that productivity (Qp) seems to diverge from day 8 to 25; however, this is purely due to cell size difference, as shown in Figure 5.

and 0.20X medium on days 3 and 4, respectively, and then full concentration for the remainder of the run. Concentration changes were made based on countering osmolality drops typical of perfusion (not shown).

The starting point for the bleed was determined by a sudden change in growth rate as the cell density increased. Initially, at the full concentration, the medium was over-bled, causing the percent viability to be too high and the cell density to be too low. This had an additional effect of increasing the cell diameter (Figure 5). After the bleed was rectified, a steady state was achieved with a viable cell density of about 90 million cells/mL for the 0.66X medium and 120 million cells/mL for the fully concentrated medium. In the steady state, protein production was over 1.7 g/L/day at the full concentration and over 1.2 g/L/day at the 0.66X concentration. For comparison, this cell line produced approximately 3 g/L protein in fed-batch culture (not shown).

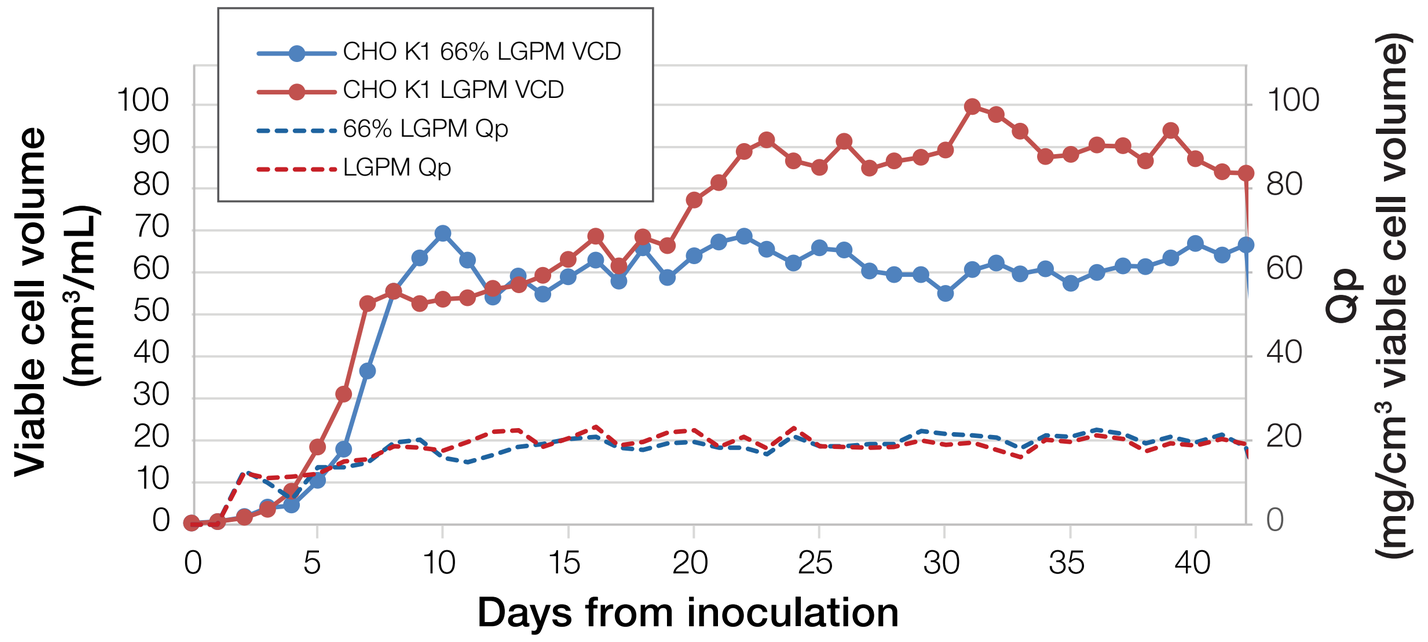

Figure 5. Viable cell volume (viable cells/mL x 4/3 x π x (average cell radius)^3) is plotted along with "true" volumetric productivity (mg product/viable cell volume).
Note: Units for Qp shown in this graph are different from typical Qp units based on VCD.

When comparing run conditions, it is important to consider cell size. Figure 5 normalizes run data with cell size, showing viable cell volume (solid lines) and productivity per viable cell volume (dashed lines). Looking at the viable cell volume being maintained between the two conditions, it can be seen that the decline in growth rate (used to determine when to start the bleed) occurred at a similar viable cell mass. Looking at productivity as milligrams of product per viable cell volume, the two conditions are almost perfectly in alignment during the entire process.

Both conditions demonstrated successful operation with HIP CHO Medium and maintained high steady-state percent viability and stable production. It was clearly demonstrated that the perfusion medium can generate a stable process in a range of concentrations. This allows use of higher concentrations to push for lower VVD and higher cell density and titer, or lower concentrations to support cell lines that need leaner medium or intentionally higher VVD.

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Additional resources

For additional resources, go to **thermofisher.com/perfusion**.

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