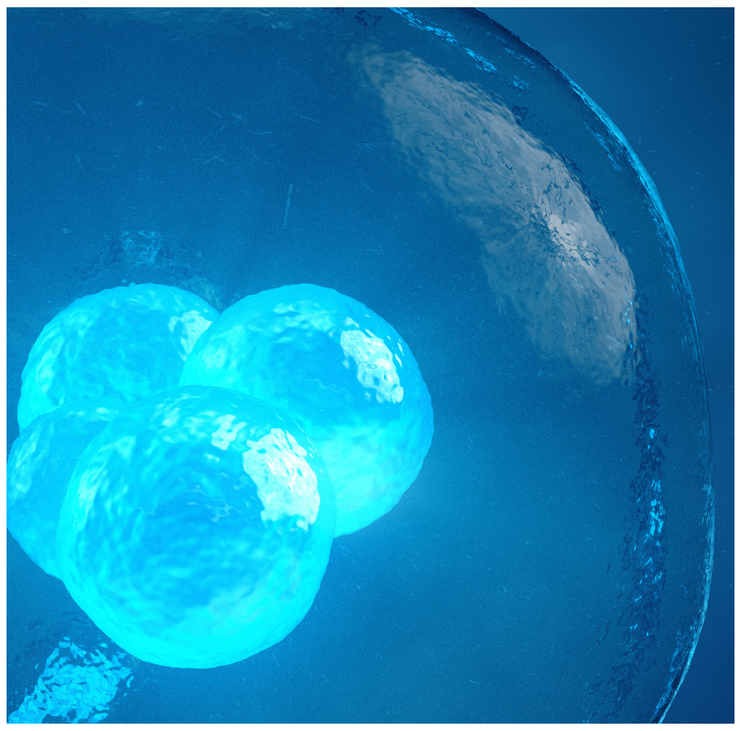
gíbco

Perfusion overview

ThermoFisher
SCIENTIFIC

Contents



What is perfusion? 3

Advantages of perfusion 4

Types of perfusion 5

What is perfusion?

TOC

In cell culture, perfusion is a process that uses a method to keep cells in a bioreactor while continuously exchanging culture medium. Fresh medium replenishes nutrients and carbon sources, while cellular waste and medium depleted of nutrients are removed. This exchange of medium is commonly expressed as the number of operating vessel volumes per day (VVD). For example, if 2 L of medium is being perfused daily into a system with a 2 L working volume, this would be expressed as 1 VVD.

For suspension culture, there are two primary cell retention methods: filtration and settling. Filtration methods, like tangential flow filtration (TFF), cycle medium from the reactor through porous hollow fibers. Cells are too large to pass through the hollow fiber membranes and hence cycle back into the reactor vessel as spent medium (permeate) flows across the membrane. Settling methods use nonturbulent flows to allow cells to settle and accumulate. This portion of the flow is cycled back to the reactor to retain cells with the rest going off as spent medium. Settling methods are generally imperfect at retaining cells, resulting in some cell removal (often referred to as bleed) in the spent medium. Retention mechanisms are selected based on the process needs; settling methods may have reduced costs and lower risk of system fouling, whereas filtration methods that allow 0.2 µm filters provide fully clarified permeate that can immediately be linked to further downstream processing.

Comparison of cell retention methods
The two methods for suspension cell culture retention, settling and filtration, have different advantages and disadvantages that need to be considered.

Settling methods
Examples:
- Passive
- Acoustic
- Centrifugation

Advantages:
- Potentially lower cost
- Potentially lower risk of system fouling

Disadvantages:
- Imperfect efficiency—some cells will be lost in spent medium
- Can be difficult to hit specific operation scales
- Passive settling may take too long and impact cell culture performance

Filtration methods
Examples:
- Tangential flow filtration (TFF)
- Alternating tangential flow filtration (ATF)

Advantages:
- Scalable
- Potential for permeate to be directly linked to downstream processing
- Perfect cell retention allows for full control of cell bleeding depending on process needs

Disadvantages:
- May require higher cost than comparable settling methods
- May increase risk of fouling

Advantages of perfusion

TOC

There are many advantages to using perfusion, including flexibility, low cost, improved quality, and speed.

Flexibility

- **High-quality production on demand**—High-quality production is achieved with a smaller footprint than with a traditional fed-batch process (both upstream and downstream). When perfusion is paired with single-use technology, it becomes easier to implement the workflow to produce what is needed when it is needed. Users can easily switch between product runs using the same equipment.
- **Scalable commitment**—Perfusion allows the end user to start with a smaller commitment and, if justified, expand quickly allowing for lower capital risk on investment.
- **Easier technology transfer**—Meeting technology transfer needs and replicating workflows is easier with a physically smaller process that has potentially fewer steps and less scale-up compared to fed-batch methods.

Low cost

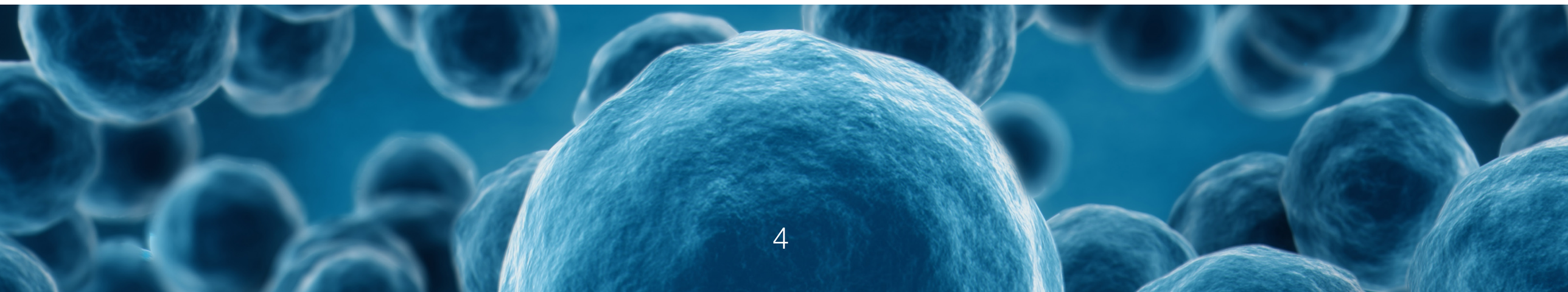
- **Simplified logistics**—Smaller equipment leads to a smaller process footprint, including capital expenditure, utilities, facility build, and cost of goods.
- **Scalable commitment**—Faster and easier facility builds allow for investing and expanding production as needed.

Improved quality

- **Improved lot control**—Option to logically define lots reduces process risk.
- **Process control**—Lower product retention time and the potential to run a continuous operation allows for better process control and minimal delay through product production, refinement, and completion.

Speed to market

- **Potential for faster production of clinical doses**—Perfusion can generate significant product volume with only minor process scale-up. This can reduce time and effort needed to generate enough doses for clinical testing.
- **Better process control**—Easier achievement of consistent quality can lead to faster process development.



Types of perfusion

In general, there are four primary operation modes for suspension cell culture perfusion: N-1 perfusion, concentrated fed-batch, intensified fed-batch, and continuous perfusion.

N-1 perfusion

Also called intensified seed train, N-1 perfusion is used to achieve high cell densities while maintaining logarithmic growth (Figure 1). This is a short-duration (usually 4–7 days) method that is used to reduce the number of vessels required in a seed train, allow for higher-density reactor seeding, or generate high-density seed banks. Productivity is not usually a consideration.

Features of N-1 perfusion

- Easy to run
- Less equipment needed
- Less characterization needed to optimize medium
- Generates high cell density quickly
- Medium exchange rate intended to "stay ahead of cell demand" to maintain logarithmic growth
- Duration of run is short (4–7 days)
- May reduce the number of vessels in the seed train
- Provides higher seeding density to production reactors
- May be used to generate high–cell density banks
- Does not require high productivity

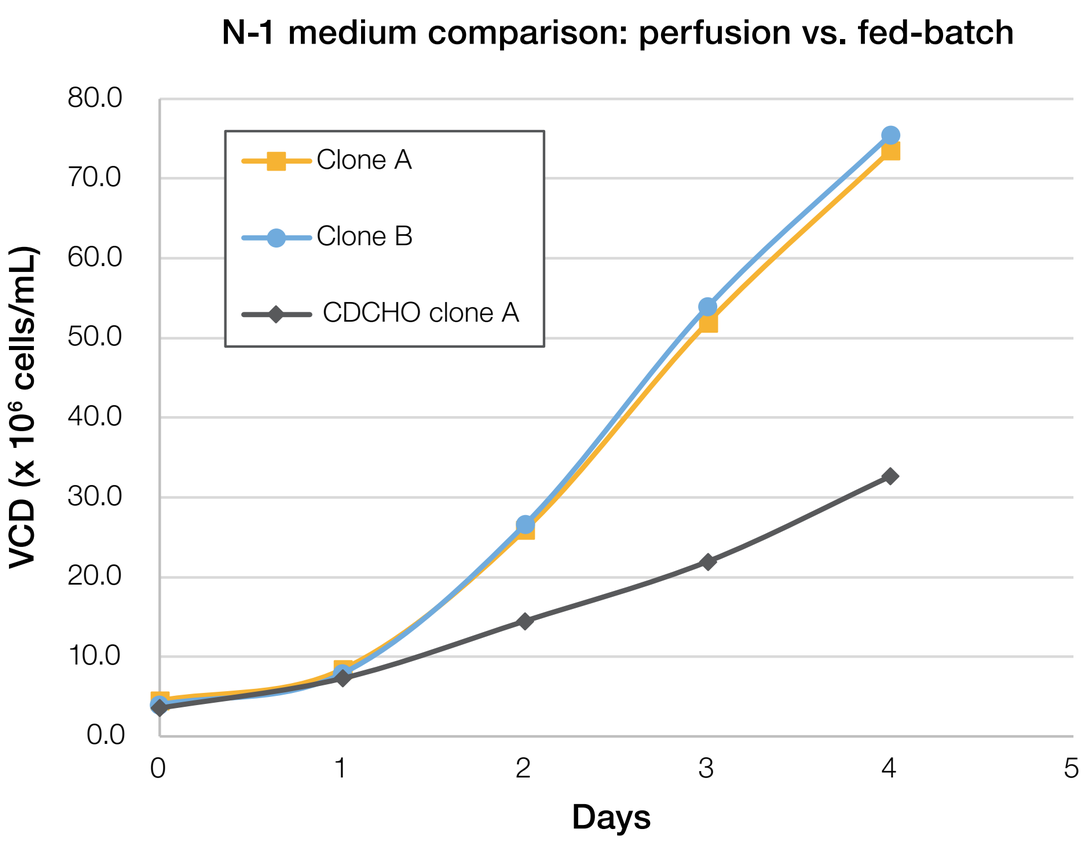


Figure 1. Operation of two clones in Gibco™ High-Intensity Perfusion CHO Medium, compared to operation in a fed-batch medium. Faster cell growth and higher viable cell density (VCD) are achieved with N-1 perfusion.

Concentrated fed-batch perfusion

With a concentrated fed-batch process, alternating tangential flow filtration (ATF) or TFF must be used as the cell retention method, as both the cells and the product are returned to the reactor throughout the run. This process allows for concentration of the product and significantly increased titer (Figure 2). Concentrated titer is ideal for stable products with low productivity in batch or fed-batch processes; the concentrated final titer allows for more productive downstream batch processing by skipping a downstream concentration step. Because product is retained in the bioreactor in a concentrated fed-batch process, perfusion product quality concerns will be typical of a fed-batch operation. Run duration is typically 14–20 days, and medium exchange rates are targeted to generate high-enough titers for downstream processing.

Features of concentrated fed-batch

- Moderate complexity
- Requires either ATF or TFF
- Requires filter pore size small enough to retain product in the vessel
- Duration of run is moderate (14–20 days)
- Requires a reasonably stable product
- Similar product quality risks as in traditional fed-batch processing
- Set medium exchange rate to target final titers for batch downstream processing

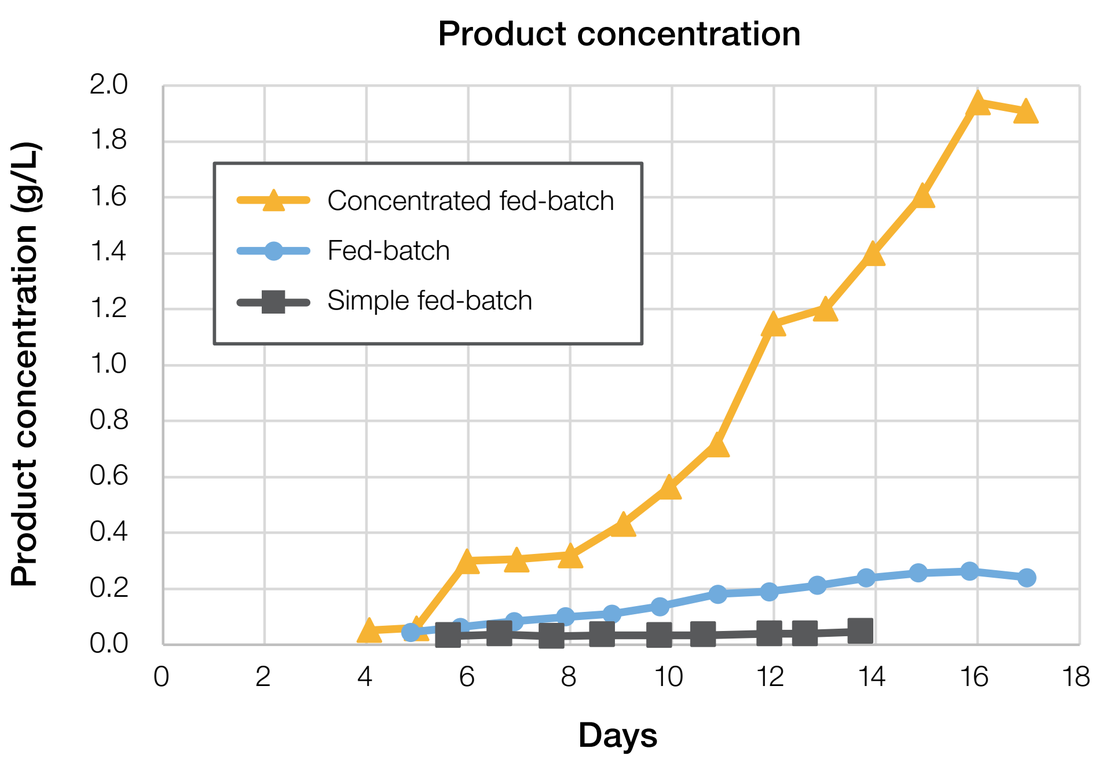
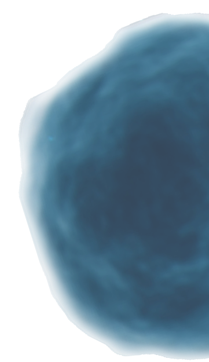
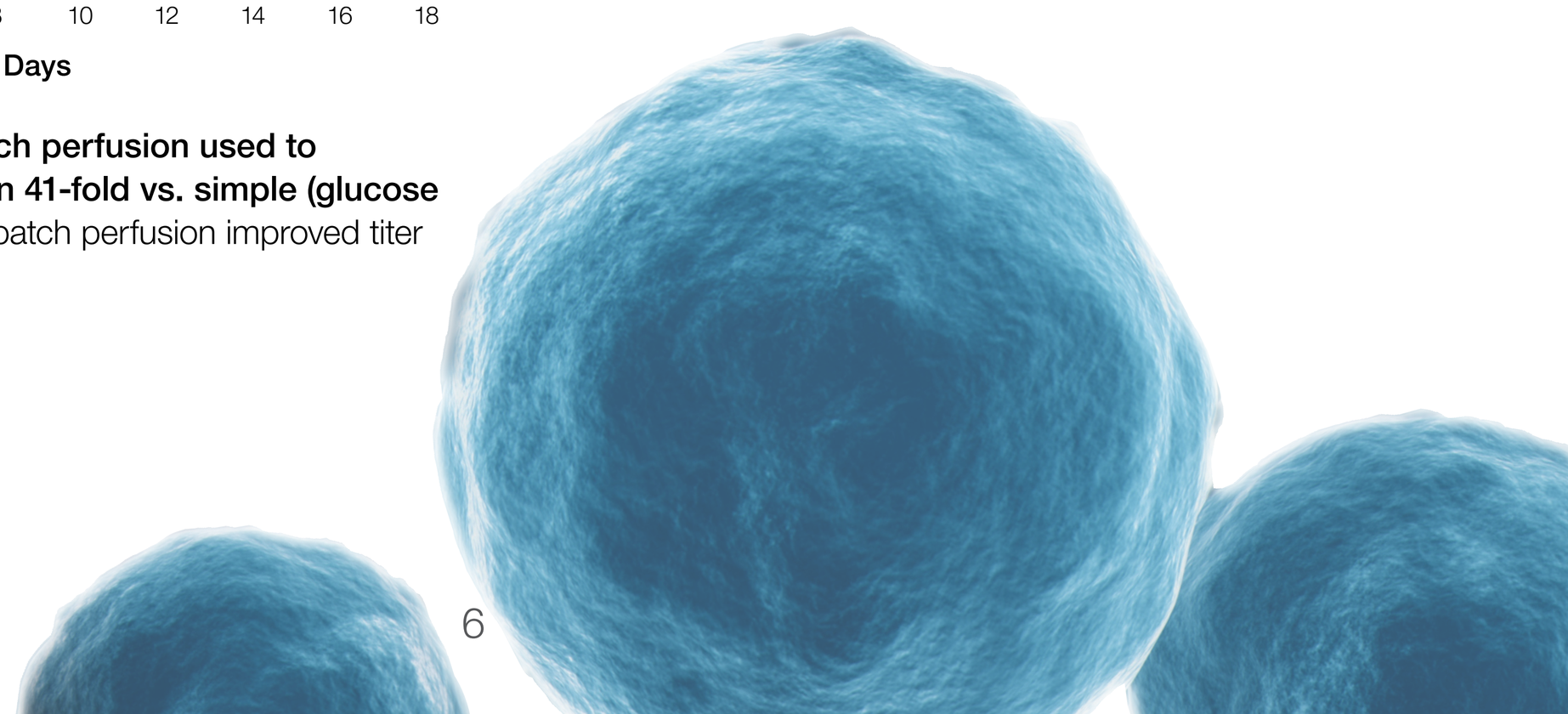


Figure 2. Concentrated fed-batch perfusion used to increase product titer more than 41-fold vs. simple (glucose only) fed-batch operation. Fed-batch perfusion improved titer more than 5-fold.





Intensified fed-batch perfusion

An intensified fed-batch process is similar to concentrated fed-batch perfusion except that the product is removed from the reactor throughout the run, making this a better option when working with more labile products and cases, where production concentration is high enough to not require a concentrating step in downstream operations. Medium exchange can lead to higher total product generated, and the shorter residence time can provide better quality control than batch operation. Constant product removal provides the option of semicontinuous downstream purification as well. If a sufficient concentration of product is achieved, ATF or TFF with 0.2 µm filters may be used to generate fully clarified permeate that can be ready to process in downstream operations. The process run time is generally slightly longer than a standard fed-batch run (16–25 days), and medium exchange rates are targeted to minimize cost per titer, often leading to a very efficient process (Figure 3).

Features of intensified fed-batch

- Moderate complexity
- Product is removed from the reactor throughout the run
- Increased total titer
- Can be used to help with a less stable product
- Lower product retention time may improve quality
- Can use TFF or ATF filters to generate clarified product stream
- Slightly longer duration compared to fed-batch run (16–25 days)
- Semicontinuous downstream or surge tank for batch downstream
- Set medium exchange rates to target minimizing cost per gram of product

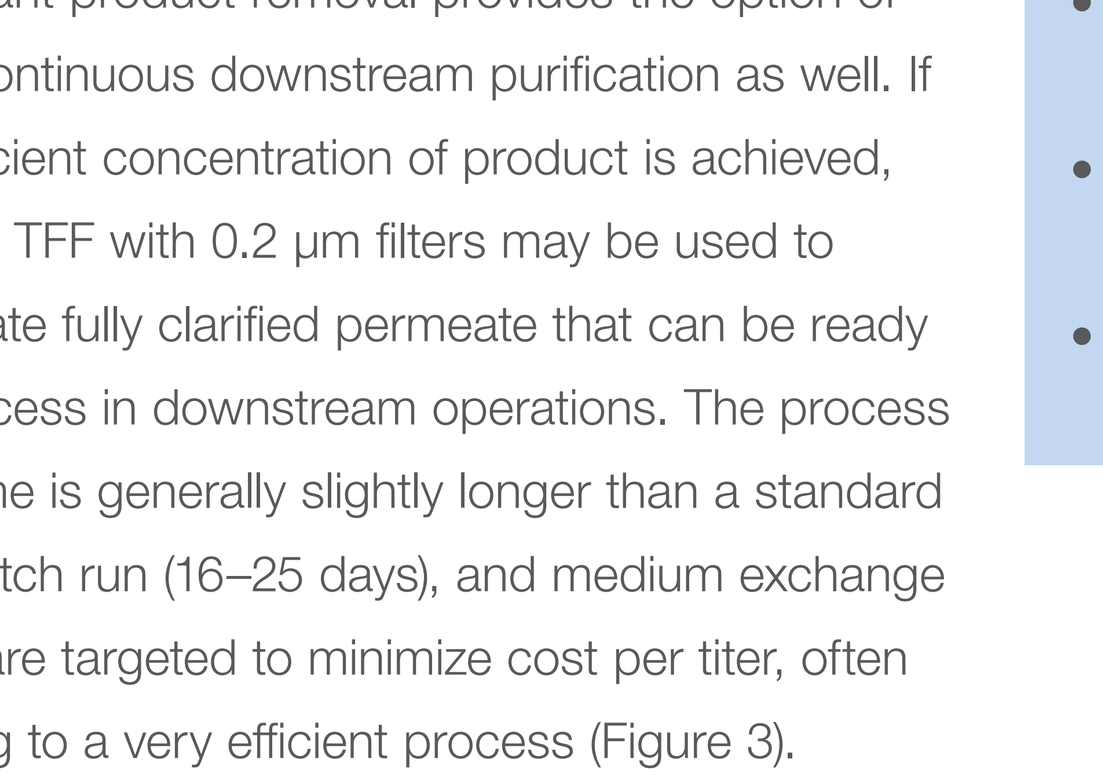
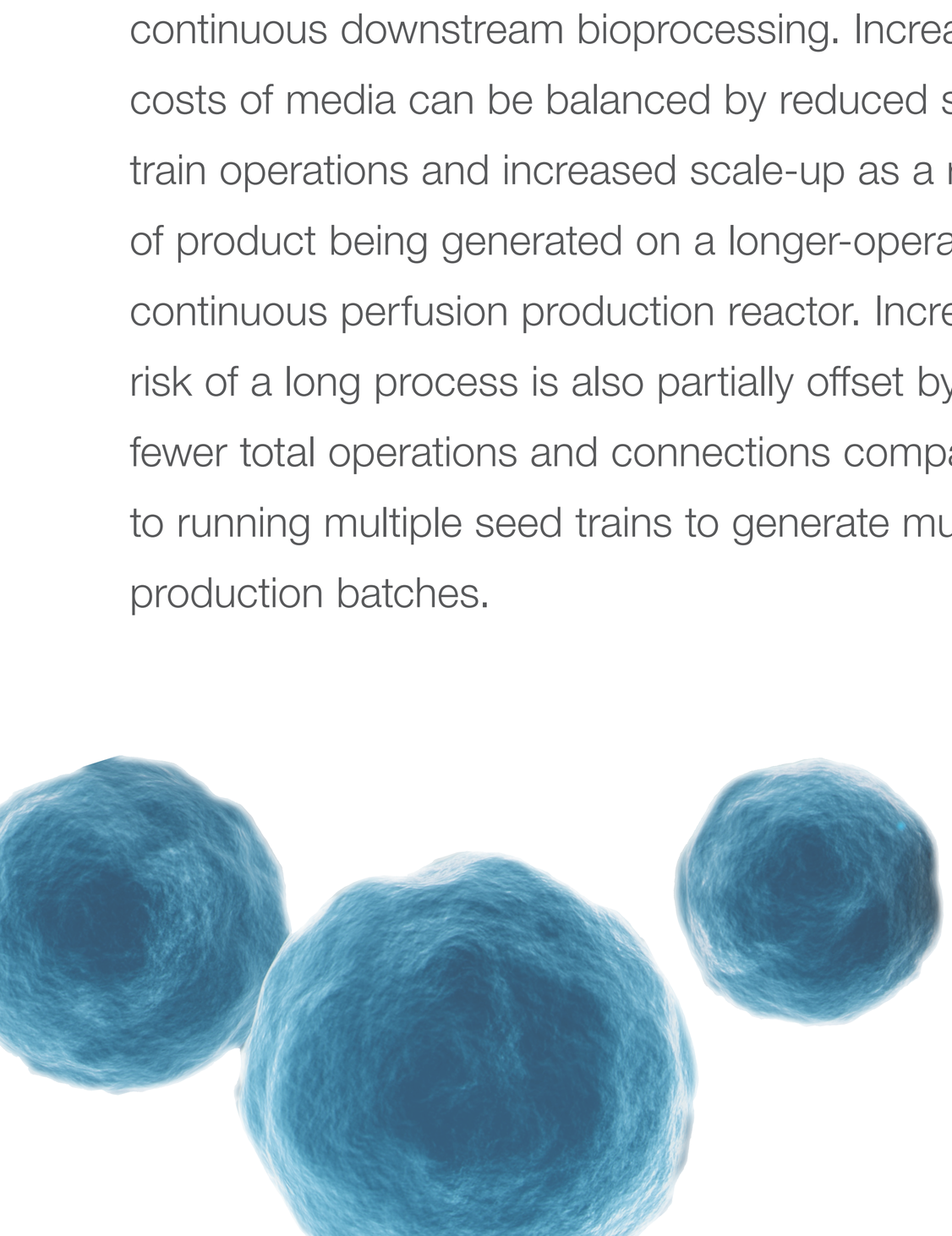


Figure 3. Intensified fed-batch run at 1 VVD. Peak VCD doubled over a typical fed-batch process. Total product production increased 7 times over that of a fed-batch run (not shown).

Continuous perfusion

The goal of continuous perfusion is to develop a process that maintains a steady state in which productivity and product quality can be sustained long-term with minimal variability. Continuous perfusion can run for 30–90 days, and an active bleed is employed to maintain a targeted viability percentage or VCD (Figure 4). Ideally, the bleed is minimized to improve process efficiency.

Like in intensified fed-batch, rates of medium exchange are optimized to maximize efficiency while maintaining tight process control. Process optimization can be time-consuming, and the long duration represents an increased risk of operational errors. It also requires a cell line with production stability that proportionately matches the targeted operating duration. Despite the technical challenges continuous perfusion is attractive, as it allows superior quality control and easy handoff to continuous downstream bioprocessing. Increased costs of media can be balanced by reduced seed train operations and increased scale-up as a result of product being generated on a longer-operating continuous perfusion production reactor. Increased risk of a long process is also partially offset by fewer total operations and connections compared to running multiple seed trains to generate multiple production batches.



Features of continuous perfusion

- Complex to operate manually, but complexity can be offset by the automation-friendliness of steady-state operation
- May use filtration to generate a clarified product stream
- Requires high cell line stability
- Long duration (30–90 days)
- Has longer process optimization times
- Generates high product titers
- Best upstream option to pair with full continuous downstream bioprocesssing
- Minimizes process footprint
- Can achieve very high product quality
- Typically, lower media efficiency compared to concentrated and intensified fed-batch (cost of goods may be offset by fewer total seed trains and production batch runs)
- A longer process equates to higher risk of individual process failure; however, this risk is relative to that of running multiple seed trains and batches, and may be mitigated by how lot size is defined

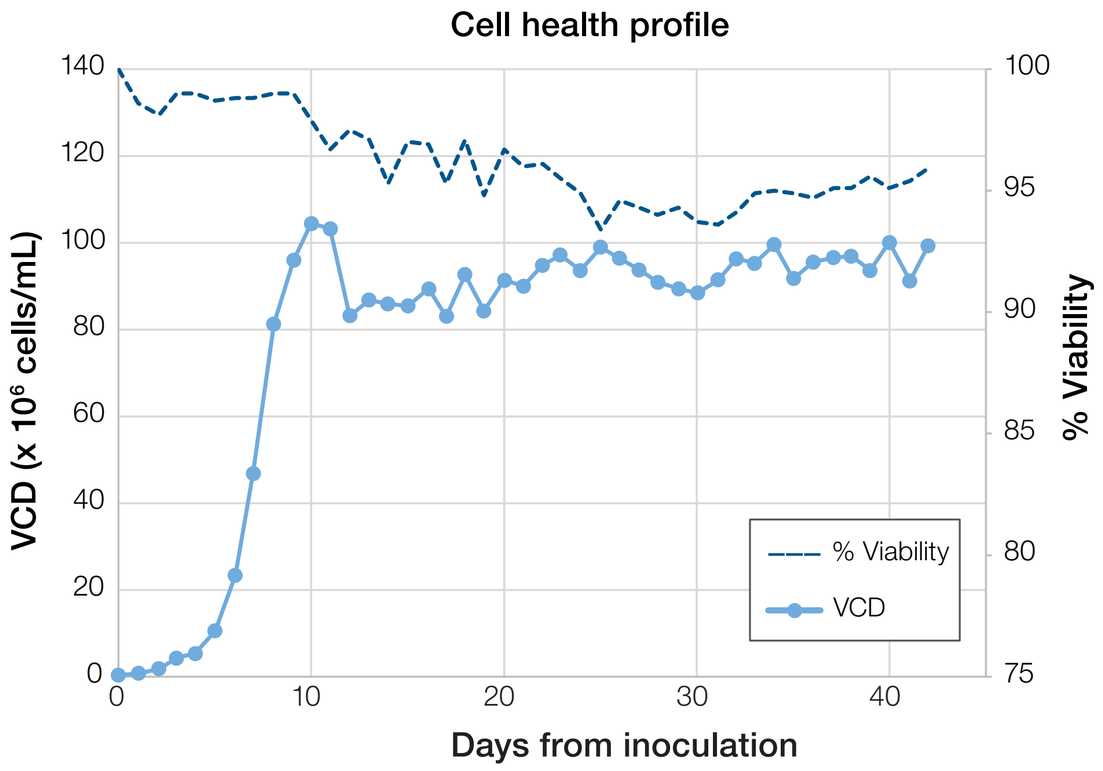


Figure 4. Continuous perfusion used to generate a stable VCD of 90 x 10^6 cells/mL and 95% viability at 1 VVD medium exchange. Steady-state production was achieved at 1.2 g/L per day (not shown). A fed-batch run with this cell line typically achieves 3.2 g/L over a 14-day production run.

gibco

Additional resources

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

Find out more at **thermofisher.com/perfusion**

ThermoFisher SCIENTIFIC