

# Understanding perfusion terminology

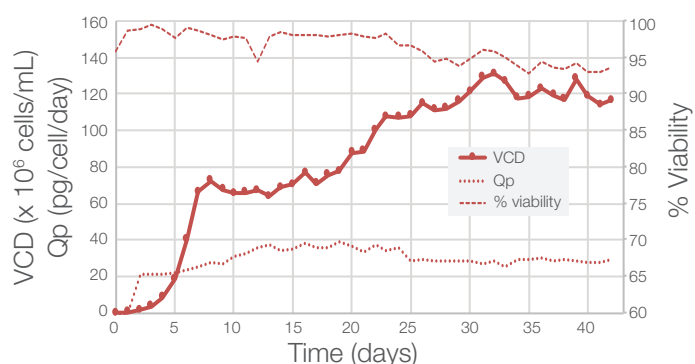
## Introduction

Over the past several years, perfusion has become an increasingly attractive process for development in the biopharmaceutical industry. It is seen as an opportunity to improve overall productivity, and unlike fed-batch culture, perfusion can be integrated into continuous bioprocessing workflows for constant product production and purification. Furthermore, perfusion processes can be extended for longer run times, compounding these increased productivity benefits. To fully evaluate whether a perfusion application will work in a production process, it is critical to have standardized terminology. This white paper explains key terms that are essential for understanding the process.

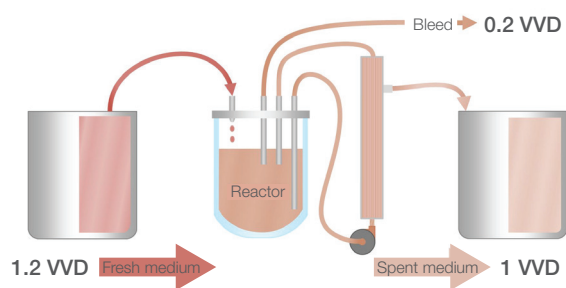
## Vessel volumes per day: medium exchange rate in perfusion culture

Perfusion-based cell culture has some key differences from fed-batch culture in both operation and terminology. For example, with fed-batch culture, concentrated feeds are provided at intervals to replenish nutrients into a fixed bioreactor. Harvesting the batch will begin when productivity stops. In a perfusion process, cells are retained inside the bioreactor and there is a constant exchange of medium—fresh medium is provided to the cells at the same rate as the spent medium (cell waste products and medium depleted of nutrients by cell metabolism) is removed. This exchange is expressed as vessel volumes per day (VVD) of medium exchanged. As an example, 2 L of medium being perfused daily into a system with a 2 L working volume would be expressed as 1 VVD (Figure 1). Reducing VVD directly reduces the amount of fresh and spent medium handled by the system and may reduce strain on cell retention devices. However, higher VVD can allow for higher sustained viable cell density (VCD), productivity, and quicker product removal, which may further increase product quality. The most common medium exchange rates are between 2 and 3 VVD.

While VVD is generally used to describe the total medium exchange rate, occasionally a publication will refer only to the spent medium flow rate when stating VVD. Additional medium may have been removed via sampling, bleed, or other mechanisms. If the quoted value is only referring to the spent medium, it will be lower than the total medium actually being exchanged (Figure 2), which could make the quoted VVD highly misleading.



**Figure 1. Example of perfusion culture at 1 VVD.** Continuous perfusion using Gibco™ High-Intensity Perfusion (HIP) CHO Medium is shown. At day 19, the operation was changed to better utilize the medium, resulting in a new steady state targeting a lower percent cell viability. The new targets were reached around day 29.



**Figure 2. Simple diagram of perfusion flow rates.** Typically, VVD refers to the total medium exchange rate. The VVD in this example would be 1.2 VVD. Sometimes, VVD will refer to only the spent medium, which can be misleading. If a bleed was used, the spent medium flow rate will be lower than the actual total medium being exchanged.

### Bleed: removal of cells from the bioreactor

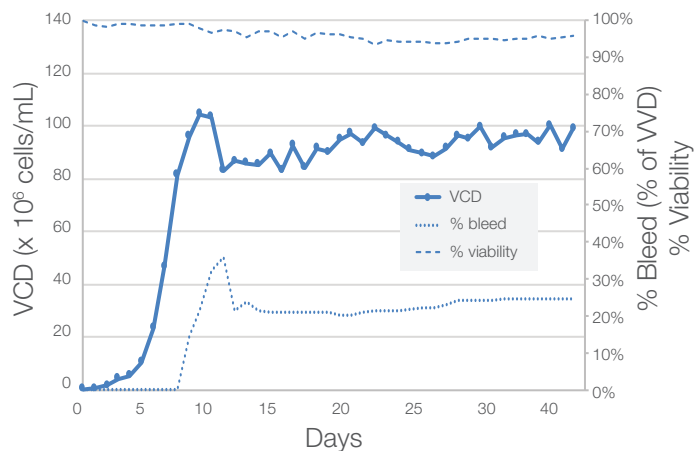
A key component of continuous perfusion is cell bleed. In continuous perfusion, and on rare occasions in intensified fed-batch perfusion, cell biomass is removed from the reactor to maintain the desired cell health and culture environment. The bleed starting point is generally determined by a sudden change in growth rate as viable cell density (VCD) increases (Figure 3). Bleed is typically described as a flow rate or percent bleed and is the percent of VVD medium flow rate that is directed toward bleed instead of spent medium. In Figure 2, the bleed would be 16.7% ( $0.2 \div 1.2$ ). Bleeds are ideally minimized to maximize process efficiency. A bleed rate can sometimes be referred to as a cell discard rate (CDR).

### Cell retention efficiency: percentage of cells maintained in a bioreactor

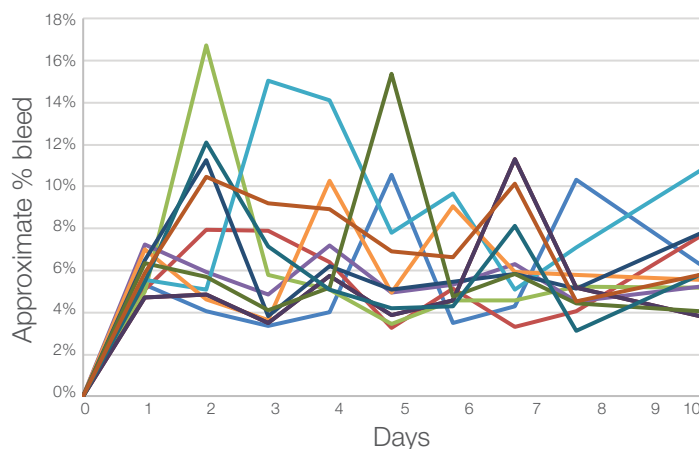
“Inherent bleed” is the percentage of cells lost in spent medium during a perfusion application; when using a settling-based cell retention method the term “% efficiency” is often used instead, and this is the percentage of cells retained in the reactor. This is the percentage of cells that are cycled back into the bioreactor versus the cells lost in the spent medium being removed. For comparison, filter-based methods such as alternating tangential flow filtration (ATF) and tangential flow filtration (TFF) have no cell loss in spent medium; they would be considered 100% efficient.

Careful consideration is necessary when interpreting the data if plans are to use two different cell retention methods in scale-down models and scale-up processes. For example, an intensified fed-batch perfusion run with a centrifuge method that is 90% efficient would effectively generate a 10% natural bleed. This will cause a slower rise in VCD and a lower peak VCD. Furthermore, it will have a longer peak with a slower drop compared to the same run repeated with a filter-based method.

In Figure 4, the approximate percent bleed is shown for 12 conditions of an Ambr™ 15 Cell Culture Bioreactor (Sartorius) running a scale-down perfusion model. The bleed is caused by a combination of a direct 3% cell loss due to daily culture sampling and spent medium removal during centrifugation. The average of these conditions is



**Figure 3. Bleed rate (percent bleed), VCD, and percent viability for a continuous perfusion application.** Initial bleed is increased to prevent excess cell density and then adjusted to maintain a target cell viability of 95%. This resulted in a steady state of about 95 million viable cells/mL.

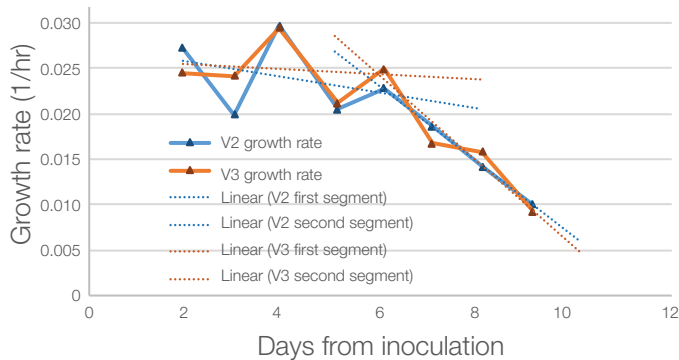


**Figure 4. Cells lost during sampling and centrifuge in a scale-down perfusion model using an Ambr 15 Cell Culture Bioreactor.** A total of 12 conditions are shown as approximate percent bleed.

typically around 6.5%, with significant day-to-day variation since it is a manual process. Daily bleed can be made more uniform by tracking the unintended cell loss in the spent medium and bleeding cells directly to allow for a consistent total cell bleed of ~10%. Cell bleed could be higher if it makes sense for the process. A 10% bleed in the scale-down model is not equivalent to a 10% continuous bleed at larger scales due to the constant growth of the cells. A 10% immediate cell loss has a larger impact than a 10% continuous cell loss in a scaled-up process.

## Cell-specific perfusion rate: medium exchange rate required for a given cell clone

The cell-specific perfusion rate (CSPR) is the medium exchange rate needed to sustain a given cell clone in a perfusion process. CSPR is most commonly expressed in picoliters per cell per day, which provides the expected medium exchange VVD per a given VCD target. There are multiple methods to estimate CSPR, from simple flask methods to running an intensified fed-batch culture and monitoring growth rate falloff with a constantly maintained medium exchange rate (Figure 5). Most of the methods enable finding the limit where a lower medium exchange rate at a given VCD will result in reduced growth rate. A higher medium exchange rate will have no measurable impact, because excess nutrients would be beyond what the current cell density can use. Therefore, these methods are designed to generate an ideal minimum CSPR. This provides guidance on how much medium exchange is needed to maintain log growth phase and ensure metabolites are not restricted. It also estimates the maximum achievable VCD given a VVD operating limit or vice versa.



**Figure 5. Daily growth rate tracked on two perfusion replicates with a constant VVD medium exchange rate.** The sudden decrease in growth rate can be used to estimate CSPR for the cell line and medium by comparing the VCD of the two runs where the growth rate decrease occurred. V2: vessel 2, V3: vessel 3.

## Considerations for CSPR

- **Specific clone**—A clone that grows to 17  $\mu\text{m}$  and has 30 pg/day production should require more medium per cell than a 12  $\mu\text{m}$  cell with 17 pg/day production. Different clones will have a different CSPR.
- **CSPR is not linear**—Cell growth rate and behavior changes with increasing cell density. It is desirable to estimate CSPR as close to final expected target operating conditions as practical during initial estimation.
- **Minimum CSPR helps keep cells in log growth phase**—This growth may be excessive relative to the acceptable parameters of a given cell run.
- **Medium formulation and cell clone nutrient requirements are different**—These requirements vary depending on how the process is run, as different metabolic pathways will be emphasized or reduced. Cell clones may behave differently with two different medium formulations at different operating cell densities relative to the medium exchange rate.

An initial CSPR estimate should be treated as guidance for process development with specific clone and medium pairing. The final, actual steady-state CSPR could differ from the initial, ideal minimum CSPR. A different medium formulation may result in both a different ideal minimum CSPR and different final operating steady-state CSPR for an optimized process.

## VCD and percent viability: operational control targets in continuous perfusion

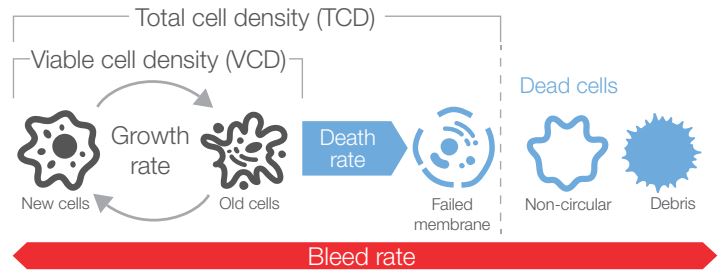
VCD and percent viability are widely used terms with respect to perfusion and fed-batch culture; however, both take on different natures as control targets for managing, optimizing, and automating a continuous perfusion culture. Percent viability and VCD can be adjusted using bleed to maintain cell culture health during continuous perfusion:

- VCD can be kept low enough to avoid excess nutrient loss or waste buildup to prevent a decrease in productivity and a surge in the cell death rate that may not be recoverable
- The culture can be pushed to a specific percent viability to maintain a consistent quality target

At steady state, VCD is constant and growth rate is balanced by death rate and cell removal rate from bleed (Figure 6). If bleed is increased, the VCD will be reduced and growth rate will increase until a new steady state is reached. At a higher constant growth rate but the same or lower death rate, there is a higher percent viability. If bleed is decreased, the VCD will increase and growth rate will decrease. If the death rate does not change, there will be a lower percent viability. This can be done until a critical point is reached where growth rate is lower than the bleed plus death rate, which will result in VCD and percent viability moving downward. To limit cell bleed but still maintain high VCD and percent viability, cell death rate needs to lower as the VCD climbs higher.

In a perfusion process, it is common to focus on the VCD for process control. Paired with CSPR, VCD can be used to automatically adjust VVD medium exchange rate during log growth phase to maximize medium efficiency. It also can reduce cell exposure to excessively high nutrients with an easier transition to a steady state. Estimating maximum VCD and expected necessary VVD helps with planning for initial estimates of necessary equipment as well as initial planned operating targets. Maintaining steady state often translates to adjusting bleed to maintain a defined VCD control target. However, this will typically result in a poorly optimized process. Looking back at how bleed works on the process, if VCD is targeted too low, bleed will be higher than necessary, excessively wasting nutrients and making the process operate at a higher percent viability than necessary. If VCD is targeted too high, bleed will be too low, risking excessive nutrient depletion. This can cause a percent viability that is too low or a risk of crashing the culture.

By comparison, if we define a target steady-state percent viability aligned to the product quality needs, the process of adjusting bleed to actively drive and maintain a percent viability will automatically push the process steady state to maintain a target quality profile while utilizing medium more efficiently. The VCD will balance to whatever can be reasonably maintained while keeping a target quality profile at the given VVD medium exchange rate with that specific clone and medium. This method is better for quick process optimization. It also can provide a fair comparison when evaluating different media and operating test conditions for a perfusion process. The results will be more meaningful on scale-up to help ensure a consistent quality profile.



**Figure 6. Relationship between VCD, total cell density, and bleed rate.**

### Viable cell volume: a superior alternative to VCD

VCD is a parameter used to provide target operations, enable automation, and help evaluate process performance and behavior. However, during a perfusion run, cell size can vary considerably. This can influence nutrient requirements, productivity, and metabolic behavior, which can impact cell culture operations. For a perfusion culture operation where VCD may be part of the control strategy, it is critical to pay attention to cell size. Viable cell volume (VCV), per the following equation, multiplies viable cell density by the average volume of the viable cells. VCV is a superior parameter to use in place of VCD, with convenient units of  $\text{mm}^3/\text{mL}$ .

$$\text{VCV} = \text{VCD} \cdot \frac{4}{3} \pi \left( \frac{\text{Cell diameter}}{2} \right)^3$$

A small increase in cell diameter from  $12 \mu\text{m}$  to  $13 \mu\text{m}$  results in a cell volume increase of 27%. An ideal minimum CSPR estimated at the smaller cell size can result in a significantly insufficient medium exchange rate. When trying to determine a cell culture steady state, VCD may appear stable, with consistent cell division rate vs. death rate, but the cell diameter may be increasing. If the cell size increase is not considered, it is possible to mistake the culture for being at steady state. In reality, the total cytoplasm the medium must support is increasing, and an eventual drop in percent viability may occur as the total cell mass requirements exceed the nutrients being supplied. By working with VCV instead of VCD, better correlations on cell culture behavior are reached, enabling more consistent control and automation.



When considering cell-specific productivity (Qp), commonly expressed as picograms of product per viable cell per day, using VCV tends to be more appropriate than VCD, as productivity often scales with total viable cytoplasm better than viable cell count. Units for specific productivity using VCV can be easily expressed as mg product per cm<sup>3</sup> VCV per day.

### Conclusion

Understanding each of the parameters discussed in this paper is crucial to understanding perfusion. Without a clear understanding of the terminology, the biopharmaceutical industry risks missing out on the opportunity to implement a perfusion process that improves overall productivity and establishes a continuous bioprocessing workflow.

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