

Upstream Intensification – Enabling Perfusion Processes with Cell Retention Technologies

Alison Dupont, Senior Development Engineer
Allyson Fournier, Applications Engineer

While delivering remarkable benefits for patients, the biopharmaceutical industry continues to experience pressure to accelerate timelines, increase flexibility to address multiple-product manufacturing and/or market demand, and significantly reduce the manufacturing cost of goods. Intensified processing is one approach that is being used to successfully address these challenges by increasing speed, reducing plant footprint, and improving productivity without compromising product quality.

Upstream processes can be intensified using a number of strategies (Figure 1):

- Through the seed train with high cell density cryopreservation and perfused seed train.
- Through production processes with high seed fed-batch, concentrated fed-batch, ultra-high VCD fed-batch, steady-state perfusion, and dynamic perfusion.

In this white paper, we explore perfusion processes and how they are becoming a viable solution.

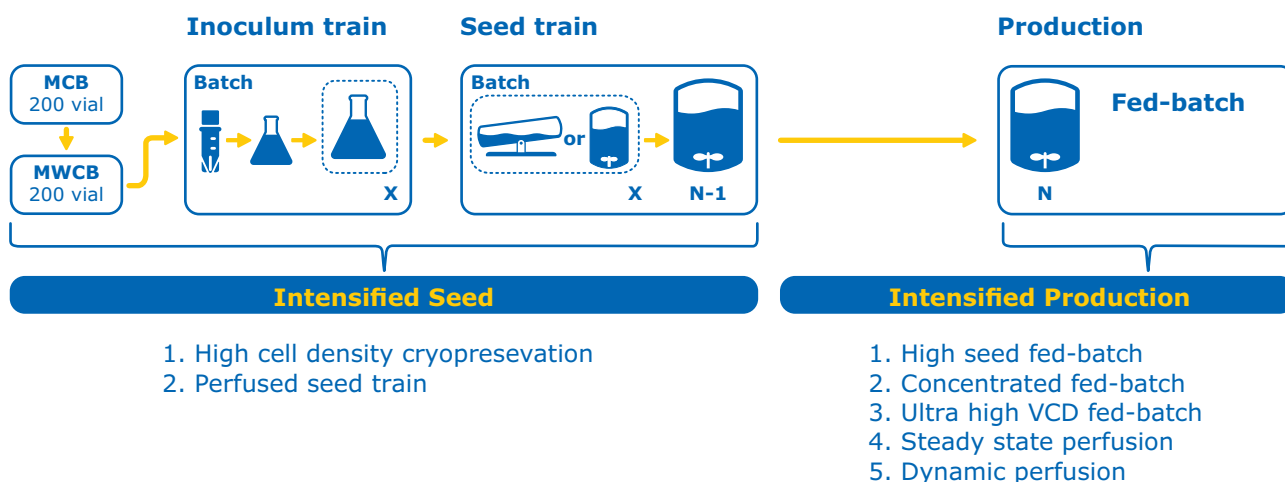


Figure 1. The seed train and production steps in upstream processing are opportunities to apply varying modes of intensification of production.

The Evolution of Upstream Processes

Upstream processes for traditional monoclonal antibody manufacturing have evolved from batch to fed-batch, with perfusion-based approaches now gaining traction. In batch processing, media is added to the bioreactor which is then inoculated. The cells consume the nutrients as they grow and expel waste, eventually reaching a limit where nutrients are depleted within the bioreactor and cells triggering apoptosis, at which point they are harvested.

With fed-batch processes, additional nutrients are added on select days to maintain growth and viability for a longer period of time, leading to higher cell densities and higher productivity. There is a limit, however, to the amount of additional nutrients that can be supplied due to the volume restriction of the bioreactor.

In contrast, perfusion processes overcome the nutrient limitations of batch and fed-batch processes by continuously providing fresh media, while simultaneously removing spent media, waste and secreted protein. This allows for significantly higher cell densities to be achieved in these processes with the overall result being higher productivity, without the need for larger bioreactors.

Historically, perfusion has been used for manufacturing of sensitive molecules that would otherwise degrade if left in the bioreactor for too long of a period or to increase the productivity of molecules expressed at very low levels. Despite the ability to increase productivity, the added risk and complexity of perfusion has prevented it from being widely adopted as the standard platform in upstream processes.

Recent advances in cell retention technologies, coupled with advancements in cell line and media development, are enabling perfusion processes to become a more viable solution.

Designing Cell Retention Technology

Figure 2 shows the components of a standard fed-batch process and the additional equipment needed to allow for continuous addition of media and removal of waste and secreted proteins. An essential component for reducing the risk and complexity of perfusion culture is a cell-retention device which allows for high cell densities and a wide range of media exchange rates, with minimal fouling and reproducible performance.

To be successful, a cell retention technology must deliver the following benefits:

- Enable robust operation over time (low fouling, good reproducibility)
- Support high cell densities and a wide range of media exchange rates
- Offer optimized process control
- Be harmless for cells (low shear, adequate pumping technology) and have a short cell residence time
- Be scalable and support all applications (N-1 and N, PD and GMP)
- Support optimum product yield

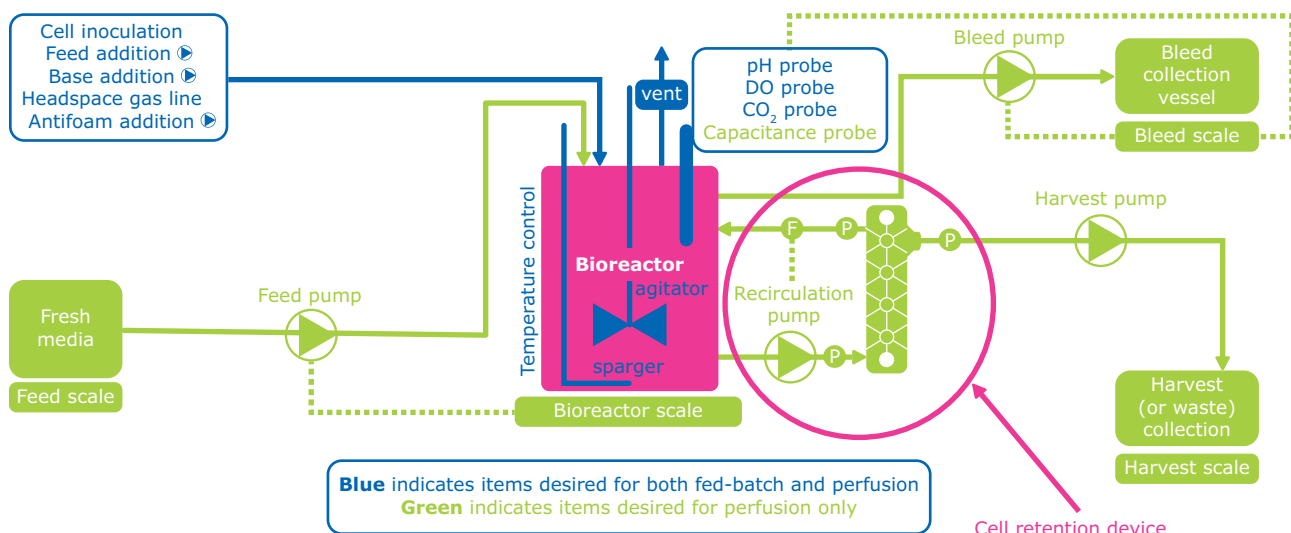


Figure 2. Components of fed-batch and perfusion cell culture; a cell retention device is critical for perfusion approaches.

Optimizing a Cell Retention Device

When designing a cell retention device, key considerations must include performance, ease of setup, scalability and cell health. Pump selection is of high importance to achieve these goals, as the risk is not only for the cells to be damaged, but also to accumulate and cause surface fouling. We investigated the use of tangential flow filtration (TFF) and optimized the membrane pore size, taking into account cells but also other impurities sizes, as we designed cell retention solutions.

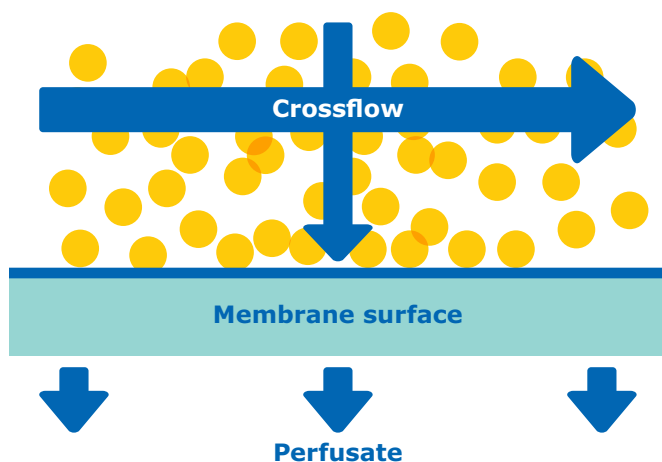


Figure 3. To prevent cells from accumulating on the membrane surface and minimize surface fouling, the crossflow rate must ideally be much higher than the perfusate rate.

In TFF, the feed from the bioreactor flows parallel to the membrane surface. This crossflow rate retains cells in the fluid path and allows them to be returned to the bioreactor. Once perfusion has started, a portion of the liquid passing through the feed channel moves through the membrane and out of the device, through the perfusate, which is controlled by a peristaltic pump. The membrane contains pores smaller than the cells and acts as a barrier to prevent cells from passing into the perfusate stream. As the perfusate rate increases, the fluid force pulling the cells to the membrane surface increases, until a point is reached where the force created by the crossflow is no longer enough to prevent cells from accumulating on the membrane surface, resulting in surface fouling of the TFF device. Ideally, the crossflow is maintained at a much higher rate than the perfusate, allowing for the device to remove a large volume of perfusate over time without surface fouling.

The bioreactor consists of a complex fluid, containing not only cells but a population of insoluble impurities with particle sizes much less than the intact cell. These insoluble impurities contribute to pore fouling of the membrane. Different membranes were evaluated within the TFF filter and the larger pore size membranes resulted in increased performance, due to a reduction in pore fouling. A number of device attributes were optimized including:

- A low-protein-binding membrane with increased pore size was selected to increase throughput and overall robustness of the device
- The channel dimensions of the device were driven by the need for scalability
- The feed-channel path length was maintained to provide scalable performance across all devices
- The throughput of the membrane influenced the requirements for the membrane area, which set the feed-channel width

Surface fouling can be controlled by increasing the crossflow rate but there is a limit due to the available single-use pumps and their flow-rate capability. Channel height has a significant impact on flow rate requirements; as channel height is reduced, the velocity of the fluid close to the membrane surface increases, allowing for the same performance to be achieved with much lower crossflow rates.

Conclusion

Perfusion processes offer key advantages to fed-batch processes and are now possible thanks to the development of a specifically designed perfusion filter and controller. We utilized TFF technologies and optimized crossflow to ensure performance, scalability and cell health. We implemented a case study to compare a conventional seed train process to a perfused N-1 seed train process, prior to a fed-batch production bioreactor (Figure 4). Cells from the perfused N-1 bioreactor, utilizing retention device technology, were used to seed a fed-batch production bioreactor at both a standard seed density and high seed density. Growth, metabolism,

and product quality trends were comparable to the control process. Importantly, the intensified upstream process resulted in greater cell-mass production in a short time and showed potential for significantly higher titers to be achieved.

Learn more

Read our application note Evaluation of Novel Cell Retention Technology and Cell Culture Media for Perfused Seed Train, a study comparing a control process to the use of perfusion process in the seed train and evaluating various process parameters (Figure 4). www.MerckMillipore.com/cellicon

Experimental condition: Perfused N-1 seed train

☐ Transfer at ~50 million cells/mL

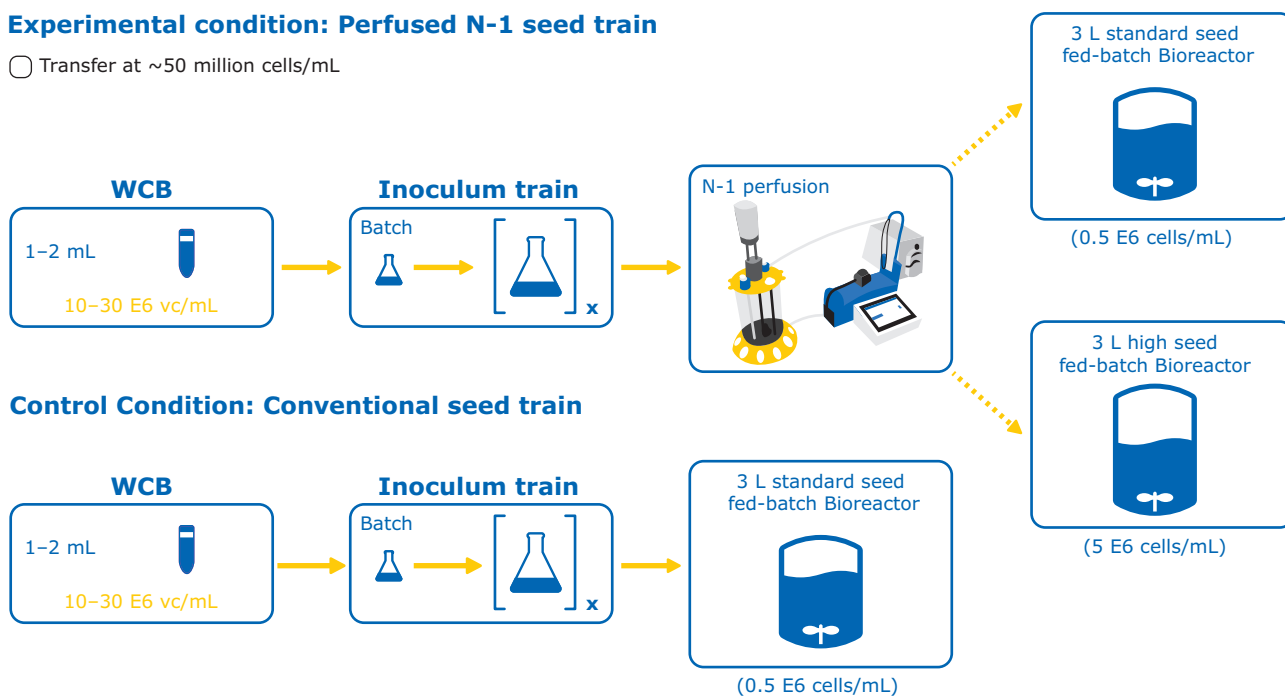


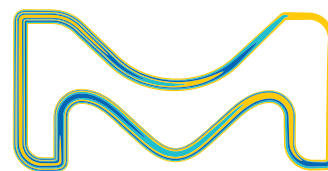
Figure 4. Our study: Experimental conditions for perfused N-1 seed train and conventional seed train.



Merck KGaA
Frankfurter Strasse 250
64293 Darmstadt
Germany

For additional information, please visit MerckMillipore.com
To place an order or receive technical assistance, please visit MerckMillipore.com/contactPS

Merck, the Vibrant M, Millipore, BioContinuum, Cellicon are trademarks of Merck KGaA, Darmstadt, Germany and/or its affiliates. All other trademarks are the property of their respective owners. Detailed information on trademarks is available via publicly accessible resources.
© 2020 Merck KGaA, Darmstadt, Germany and/or its affiliates. All Rights Reserved.



Lit. No. MK_WP5880EN Ver 1.0
05/2020