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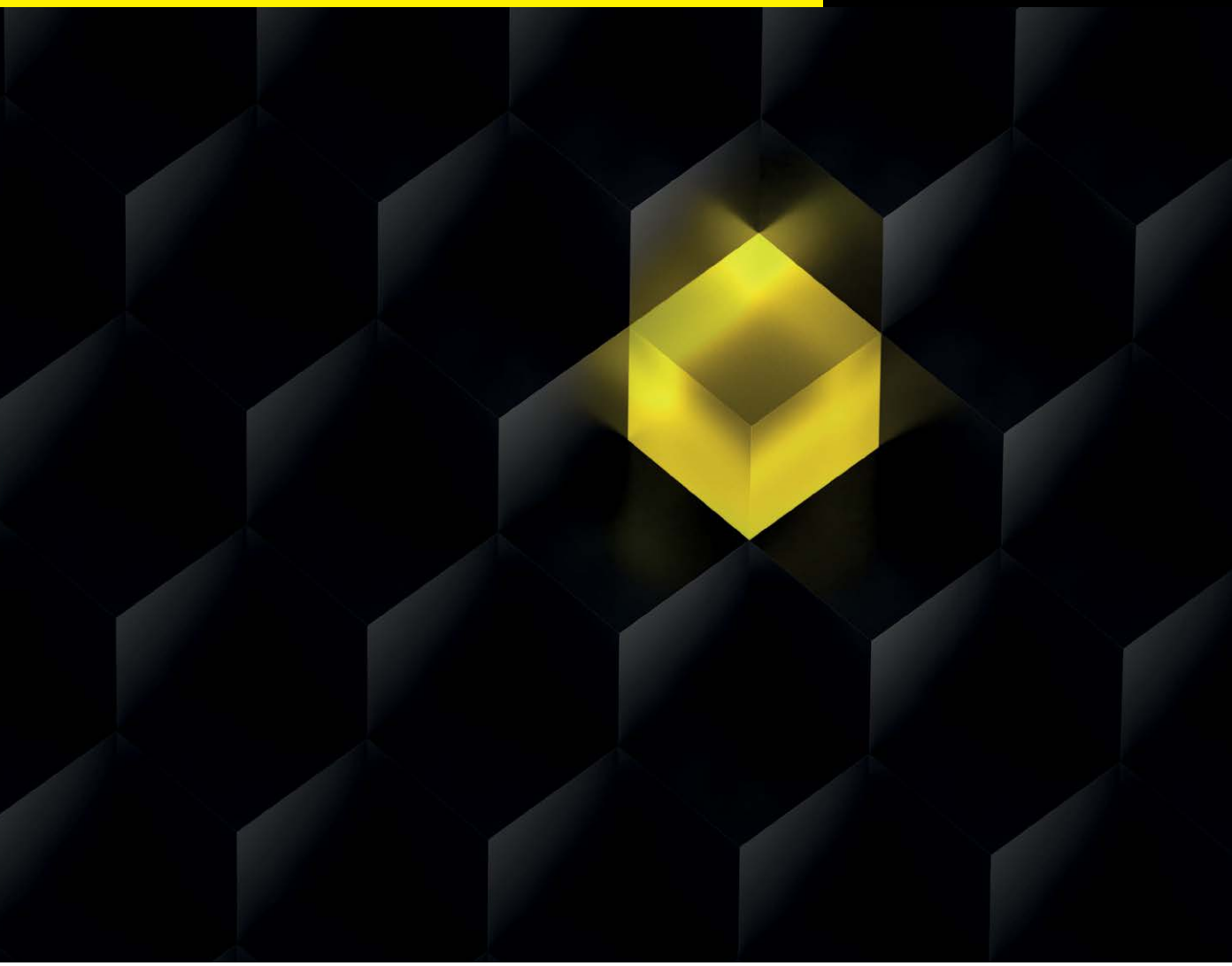


A Strategic Approach To Selecting the Optimal Process Intensification Scenario

BioProcess International

A Strategic Approach To Selecting the Optimal Process Intensification Scenario

Priyanka Gupta and Katy McLaughlin

Current demands placed on the biopharmaceutical industry are pushing manufacturers toward intensified processing. *Process intensification* (PI) describes an overall approach that modifies unit operations or an entire manufacturing process to optimize efficiency. Biomanufacturers can measure improvements by a number of approaches, including reducing operation costs, speeding up production, increasing yields, and enhancing flexibility **(1).**

Intensification strategies can be applied to individual unit operations or throughout an entire facility or process, using an end-to-end approach. The three intensification scenarios typically used in upstream processing are seed-train intensification (usually at the $N - 1$ stage), concentrated fed-batch production, and dynamic perfusion (at the production bioreactor stage) (Figure 1 and Table 1). In downstream processes, intensification strategies typically involve moving from single-column to multicolumn chromatography (MCC).

Here, we discuss how adopting single-use intensified manufacturing satisfies key business drivers. We then provide a process-decision matrix to help simplify the implementation of PI in new and existing facilities. Finally, we present an example illustrating how upstream PI can increase productivity and reduce costs without compromising quality **(2)**.

Process Intensification: A Decision Matrix

Benefits of Intensified Production: The philosophy of PI creates improvements that help manufacturers keep up with growing demands, allowing them to stay ahead of their global competitors.

First, by lowering the need for high upfront investment, PI helps mitigate the risk of failure during early biopharmaceutical development when it is not always clear which molecules will make it to market. Therefore, when building a facility to support production of a new biologic, it is important to consider options that require low upfront investment and limited expenditure on large equipment (such as multiple commercial-scale bioreactors). With PI, you can limit the amount of equipment needed to build a productive facility (Table 1).

In the highly competitive biopharmaceutical space, the first product to market has a significant advantage. PI can reduce the time it takes to get into clinical studies and/or commercial sales by accelerating development. The build-out time for a good manufacturing practice (GMP)–compliant facility is typically around four years, reduced to two years or less when PI strategies are implemented (Table 1).

Intensification also can reduce a facility footprint by 50–70%, creating a smaller, more efficient facility (Table 1). That can help developers save on overhead costs such as energy use and facility maintenance. A reduced footprint helps companies achieve sustainability targets, such as by lowering the process mass intensity index (PMI), electricity use, and water consumption. Such changes reduce the overall carbon footprint, an increasingly important factor in the current environment.

Finally, PI can increase productivity by two- to threefold (Table 1). The enhanced efficiency reduces the cost of goods by up to 30% or more, depending on the combination of approaches that are implemented throughout the process.

Process Decision Matrix: Once a drug developer has decided that intensification can benefit its facility, it must select the scenario(s) most compatible with its processes. Decision-making will be shaped by many factors, including the drug candidate, market, facility, and operations; there is no universal solution. Below is a simplified framework for selecting an optimal strategy for your facility.

Facility Throughput — 300 kg/Year or Less: Usually, the first driver to begin the decision-making process will be facility throughput (Figure 1). For facilities where throughput is under 300 kg/year, seed-train intensification or dynamic perfusion will typically be the optimal PI approaches.

An $N - 1$ perfusion, high-inoculum fed batch can increase productivity by up to twofold and can be implemented easily into an existing process, making it an excellent transition into the philosophies of PI.

Table 1: Comparing different upstream process-intensification (PI) strategies with standard fed-batch production, in which harvest happens only at the end of production; *N* – 1 perfusion (seed-train intensification) reduces the time needed to reach the cell densities required to populate a production bioreactor. Concentrated fed-batch production involves continuous circulation of media, but the product is harvested only at the end of a procedure. Finally, dynamic perfusion involves continuous feed and harvest of the product through microfiltration.

Benefits/Disadvantages Compared with Fed Batch	*N* – 1 Perfusion High-Inoculum Fed-Batch	Concentrated Fed-Batch	Dynamic Perfusion
Similar throughputs with single use and with 10-kL stainless steel	—	Yes	Yes
Productivity increase*	Up to ×2	Up to ×4	Up to ×5 in 12–20 days
Culture time saving	Up to 5 days in seed train	Up to 10 days in seed train **	Up to 10 days in seed train **
Investment CoGS	Lower	Lower	Lower
Media use	Slightly higher	High	High
Alternating tangential flow (ATF)	Yes, microfiltration	Yes, ultrafiltration	Yes, microfiltration
Batch scheduling	Short batches, flexible USP to DSP scheduling (multiproduct)	Short batches, flexible USP to DSP scheduling (multiproduct)	Less flexible, longer batches, DSP 1:1 connected to perfusion permeate

*typical numbers **with 5-mL HCD vials and RM perfusion for *N* – 2 and *N* – 1

Additionally, the increase in media consumption is modest (Table 1) (**3**).

Dynamic perfusion, followed by MCC in the downstream process, has the potential to increase productivity by up to five-fold in under three weeks (Table 1), and can significantly reduce facility footprint. However, dynamic perfusion will increase media use by over 1,000%, which will be an important consideration in facility design (**3**).

Facility Throughput — 500 kg/Year or More: Where throughput is over 500 kg per year, manufacturers will benefit most from concentrated fed-batch or *N* – 1 perfusion. Concentrated fed-batch is highly productive (up to 4× increase) and flexible, making it suitable for multiproduct facilities. However, it requires significantly more culture media than standard fed-batch production does (Table 1) (**3**). As discussed for low-throughput processes, *N* – 1 perfusion also increases productivity (about 2×) with limited process disruptions and upfront investment. Beyond increasing overall productivity, applying PI strategies to high-throughput processes can reduce facility footprint by 50% or more, significantly improving efficiency.

Facility Requirements: After considering facility throughput, manufacturers will need to consider how PI fits in with their facilities and operations.

Intensification in Existing Facilities: Approaches to implementing PI will differ between new and existing facilities. Facilities that are not yet in operation have more options because designers do not need to consider how changes affect ongoing production.

Seed-train intensification is an effective strategy that manufacturers use to prevent the need for significant changes to their existing operations and facility set ups. Although *N* – 1 perfusion increases upstream productivity, it does not affect product titers enough to require significant changes to downstream procedures. Seed-train intensification also will not change media and buffer requirements dramatically, does not involve additional expertise, and can be implemented seamlessly into an existing facility.

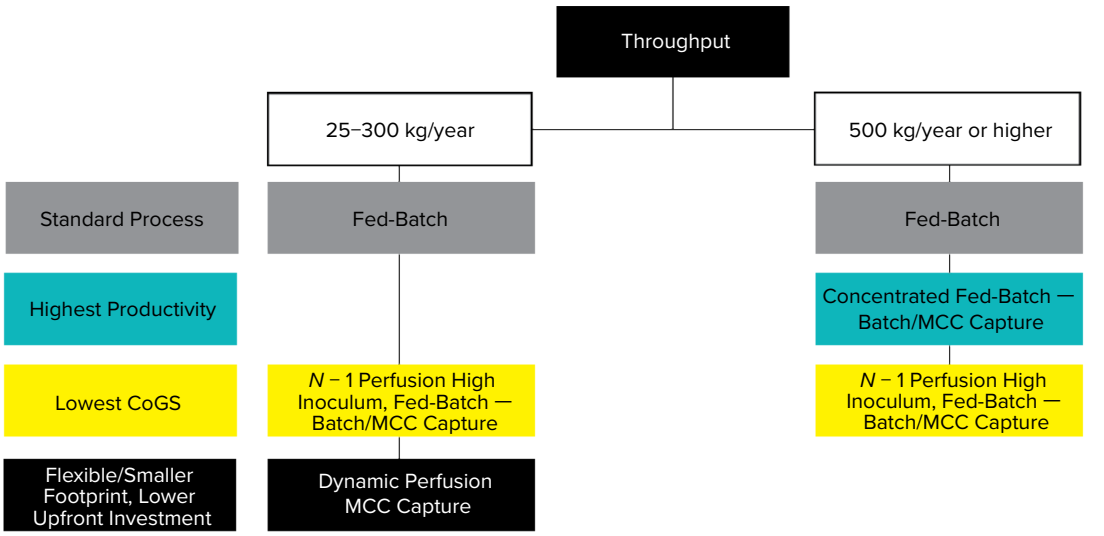
Converting to a Single-Use Facility: As current trends push manufacturers toward intensified bioprocesses, the same pressures drive facilities toward single-use (SU) manufacturing. Comprehensive SU platforms promote consistency and robustness in the process while providing the flexibility needed to respond to changes quickly. A company using traditional stainless-steel equipment might want to combine the philosophies of PI with SU technologies to create a next-generation facility.

Concentrated fed-batch production could be an excellent strategy to move away from stainless-steel facilities toward SU platforms. This strategy is most suitable for high-throughput production and offers flexibility for multiproduct manufacturing.

Sensitive Modalities: Many emerging modalities are sensitive biological molecules, such as bispecific monoclonal antibodies (bsAbs), which require adjustments to the production process for traditional biotherapies. For example, some modalities are not compatible with a standard fed-batch process in production.

Dynamic perfusion is a superior choice for unstable or difficult-to-express molecules. Also compatible with relatively small facilities implementing a modular

Figure 1: Process-decision criteria for PI implementation are included in a flow chart to simplify the selection of an intensification strategy. Upstream approaches include *N* – 1 perfusion high inoculum fed-batch, concentrated fed-batch, and dynamic perfusion. Downstream approaches include multicolumn chromatography (MCC).



approach to PI, dynamic perfusion offers flexibility for manufacturing multiple molecule types.

Intensified Seed-Train Strategy for Effective Scale-Up of Biologics Manufacturing

Seed Intensification — Proof of Concept: Seed-train intensification is an ideal first step in exploring the benefits of PI. Focusing on the seed train offers a significant increase in productivity without making substantial changes to the existing facility set-up.

The following article, first published in *BioProcess International* in December 2020, is a proof-of-concept study demonstrating the feasibility and value of seed-train intensification. Using Chinese hamster ovary (CHO) cell cultures grown in single-use bioreactors, our researchers investigate the feasibility of populating a production bioreactor with high-density cell cultures to increase the scale-up efficiency of a pilot bioprocess. They then examine the impact of their seed-train intensification strategy on critical quality attributes of the produced biologic.

Simplifying Intensification

The development and manufacture of biotherapeutics is a time-consuming, expensive, and often high-risk process. Strategies to speed up development and production, reduce costs, and promote a robust process are necessary to remain competitive.

Robust PI solutions from early development to commercial production help meet industry demands and create more sustainable, productive, and flexible facilities. Typically, upstream PI scenarios are the first to be explored.

The benefits of seed-train intensification are also backed up by experimental data. In the following study, a SU production bioreactor was populated with high-density cultures of CHO cells, facilitating a 30× reduction in inoculation volume. The intensified process generated cultures and products with characteristics that were comparable to standard fed-batch procedures.

Deciding which strategy to implement can be a barrier to integrating PI in new and existing facilities. However, following a simplified framework can help manufacturers tackle the selection process and quickly reap the benefits of PI philosophies in their facilities.

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Intensified Seed Train Strategy for Faster, Cost-Effective Scale-Up of Biologics Manufacturing

Jens-Christoph Matuszczyk, Johannes Lemke, Markus Schulze, Sabrina Janoschek, Gerben Zijlstra, and Gerhard Greller

The high costs of and limits on global accessibility of biologics such as monoclonal antibodies (MAbs) are focusing the biopharmaceutical industry's attention on strategies for rapid, economical development of such therapies. Process intensification is one approach to help shorten manufacturing timelines and reduce cost of goods (CoG) (**1, 2**). Today, process intensification in upstream cell culture enables biologics manufacturing in facilities with smaller footprints and lower scale-up volumes than was possible before. Intensified processing of Chinese hamster ovary (CHO) clones that produce MAbs is being developed in the seed train of upstream cell cultures (**3**) to support generation of high–cell-density (HCD) cell banks and processes to reduce plant size, capital investment, and overhead costs while increasing productivity.

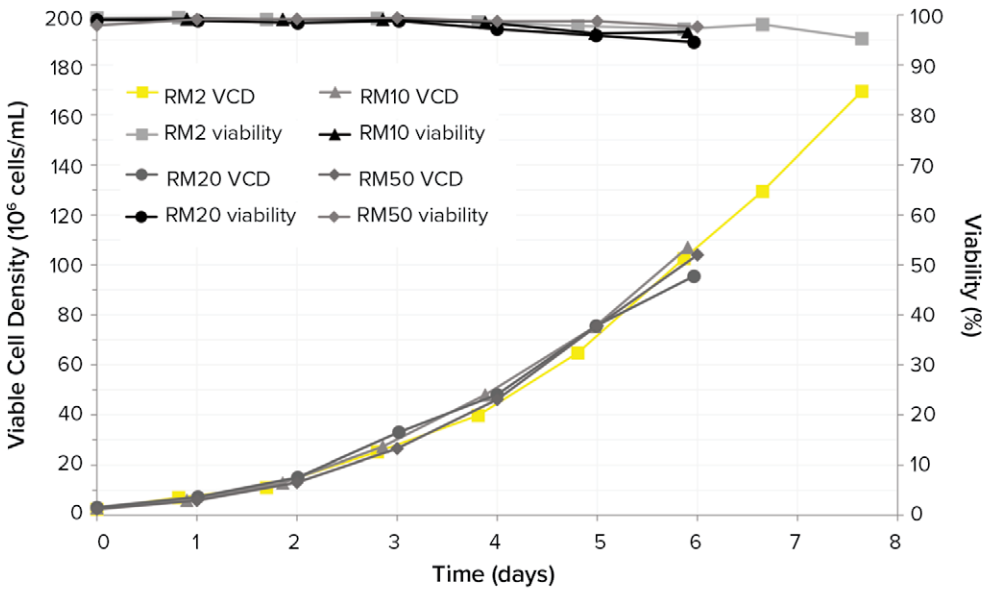
In traditional practice, MAb manufacturing relies on seed ratios of ~1:5 (**3**) with an initial inoculum of 0.3 × 10^6 cells/mL (**4**) using a seed train of five to eight transfer steps. Standard seed trains typically begin with shake flasks in the postthaw expansion (N – 8 to N – 5) and then move through a rocking-motion (RM) 5–50-L bioreactor (N – 4 to N – 3) to seed a series of N – 2 to N – 1 stirred-tank (STR) bioreactors (200 L and 500 L) ending in a production culture (N) with a volume of 2,000 L. Adopting an intensified cell culture approach at N – 4 to N – 1 by, for example, using 2D RM bioreactors in perfusion mode (where cells stay in the exponential growth phase throughout the entire culture) could increase viable cell densities to 100 × 10^6 cells/mL and drive seed ratios of >1:100. That allows skipping of process-transfer steps. For example, HCD cell banks can be thawed directly and inoculated into RM bioreactors at higher volumes (0.5–2 L depending on the HCD cell-bank volume), thus bypassing cell-culture expansion in shake flasks and the potential errors and contamination issues associated with manual handling of flasks. Also, with perfusion in the N – 1 seed train, cells can reach higher densities for inoculating a pilot or production bioreactor directly without process transfer through multiple intermediate-scale STR bioreactors. That reduces both the number of steps and the total number of seed-train days, thus reducing initial investment and operational CoG. However, not all CHO clones that produce MAbs can be cultured in intensified processing because high cell densities can compromise protein expression levels and genetic stability (**5**).

Our goal was to explore the possibility of culturing a CHO clone that expresses a commercial MAb therapeutic in HCD for an intensified, scalable cell-culture seed-train protocol. We investigated whether single-use RM perfusion bioreactors (working volume 1–25 L) could achieve a reproducible cell culture with at least 100 × 10^6/mL viable cell density (VCD) at different working volumes. The HCD culture was monitored using process analytical technology (PAT) at all scales. When the culture in the RM bioreactor (1-L working volume) reached the required HCD, a culture volume was used to seed a pilot-scale STR single-use bioreactor (200 L) automatically, which then ran in fed-batch mode. During the pilot run, we measured cell density and viability along with the MAb's critical quality attributes (CQAs), then compared those results with the same MAb produced through a standard seed train. The results established whether a HCD perfusion seed train can inoculate a standard manufacturing N-stage bioreactor to reduce the number of transfer steps and whether it is possible to use a PAT-based control regime for automated inoculation of N-stage processes to reduce inoculation volumes.

Materials and Methods

Cell Line: We chose a Cellca CHO-DG44 cell line expressing a commercially available human MAb to investigate the practicality of using HCD perfusion culture to inoculate a pilot-scale bioreactor. The cell line, with its associated media and cell-culture parameters, was selected for proof-of-concept studies

Figure 1: Viable cell density (VCD) and viability of Chinese hamster ovary (CHO) cells cultured in RM perfusion bioreactors with working volumes from 2 L to 50 L



because the fed-batch process used to produce this MAb has been well-characterized by Sartorius.

Bioreactors: The Biostat RM 20/50 bioreactor (Sartorius) was chosen for seed-train studies because it continuously renews the cell-culture medium, enabling mass transfer between the headspace and medium for efficient, low-shear mixing. The system was used with single-use Flexsafe RM perfusion bags (1–25-L working volume) constructed of pharmaceutical grade low-density polyethylene, which is used throughout the entire range of RM and STR bioreactor bags to ensure consistent quality. The bags each feature an integrated perfusion membrane at the bottom, forming a compartment for removing cell-free media during the perfusion process for minimal loss of or damage to the cells. The RM perfusion bioreactors scale from 2 L to 50 L (working volume 1 L–25 L) using a common RM bioreactor design to simplify process transfer.

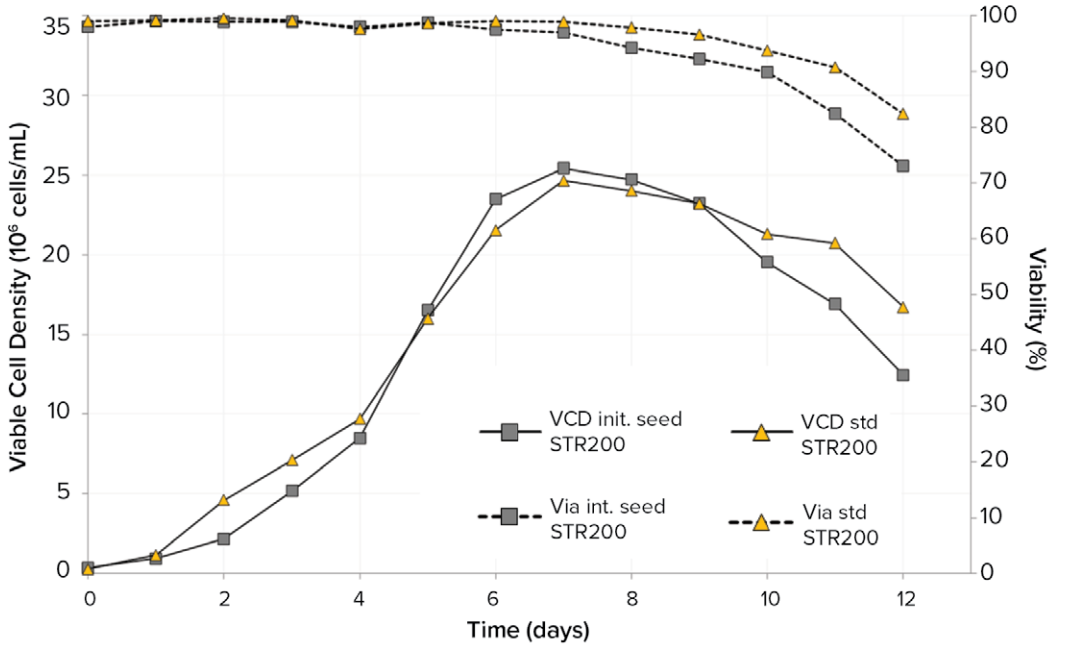
A Biostat STR bioreactor (200 L) with single-use Flexsafe STR bags was used as the pilot-scale single-use bioreactor. The Sartorius STR bioreactor range scales from 50 L to 2,000 L and uses the same established bioreactor design principles as stainless-steel bioreactors. All bags have similar geometries across scales (**5**) to facilitate process transfer and scale-up (**4**).

Proof-of-Concept Studies

Seed Train Study: To demonstrate the performance and scalability of cultures in RM perfusion bioreactor bags, we cultured CHO cells for six days in perfusion bioreactor bags of 2 L, 10 L, 20 L, and 50 L (working volumes of 1 L, 5 L, 10 L, and 25 L) using proprietary media and feeds at a minimum cell-specific perfusion rate (CSPR) of 50 PI (cells/day). The cells were cultured at 36.8 °C, pH 6.95, and DO 60% (maintained by increasing the rocking rate at a 10° angle). On-line PAT sensors, including the BioPAT ViaMass sensor, were used to monitor and control the culture's VCD and viability and enable automatic feeding. The 2-L culture was cultivated for an additional two days under perfusion conditions to determine a maximum VCD.

Pilot Bioreactor Studies: A VCD model from the RM perfusion bioreactor was created from the signal from the BioPAT ViaMass sensor. With this model, the VCD was measured on-line constantly, and when cells reached a trigger point of 100×10^6 cells/mL, a cell inoculum volume calculated to produce an initial cell density of $0.33 \pm 0.04 \times 10^6$ cells/mL was transferred automatically from the RM perfusion bioreactor to the pilot scale STR bioreactor. Cells cultured in the perfusion bioreactor (400 mL at approximately 100×10^6 cells/mL) were used to seed a Biostat STR 200-L single-use bioreactor. Cells were cultured for 12 days using proprietary media and feeds at 36.8 °C, pH 7.1, and DO 60%. Feeds were added after a three-day batch phase on day four. VCD and cell viability data were measured and collected by the BioPAT ViaMass sensor. A cell-free supernatant was sampled for N-glycan profiling to determine MAb product quality

Figure 2 Comparison of VCD and viability of CHO cells in 200-L pilot-scale fed-batch culture inoculated with cells from standard or intensified seed trains



and titer. The results were compared with historical data from a standard seed train for which cells were cultured in standard Biostat RM 20/50 bags (14 L at approximately 3 × 10^6 cells/mL) and used to inoculate a Biostat STR 200 L single-use bioreactor.

Results and Discussion

Performance of Seed Train: The results (Figure 1) show that in six days, the RM perfusion bioreactors can grow CHO cell cultures with >90% viability and with high cell densities of >100 × 10^6 cells/mL. That represents a 33-fold increase in VCD compared with an inoculum from a standard RM bioreactor. Over eight days, in a 2-L bag, these perfusion bioreactors support cell culture to VCDs of 170 × 10^6 cells/mL with >90% viability, demonstrating that cellular oxygen demand can be supported at such high cell densities.

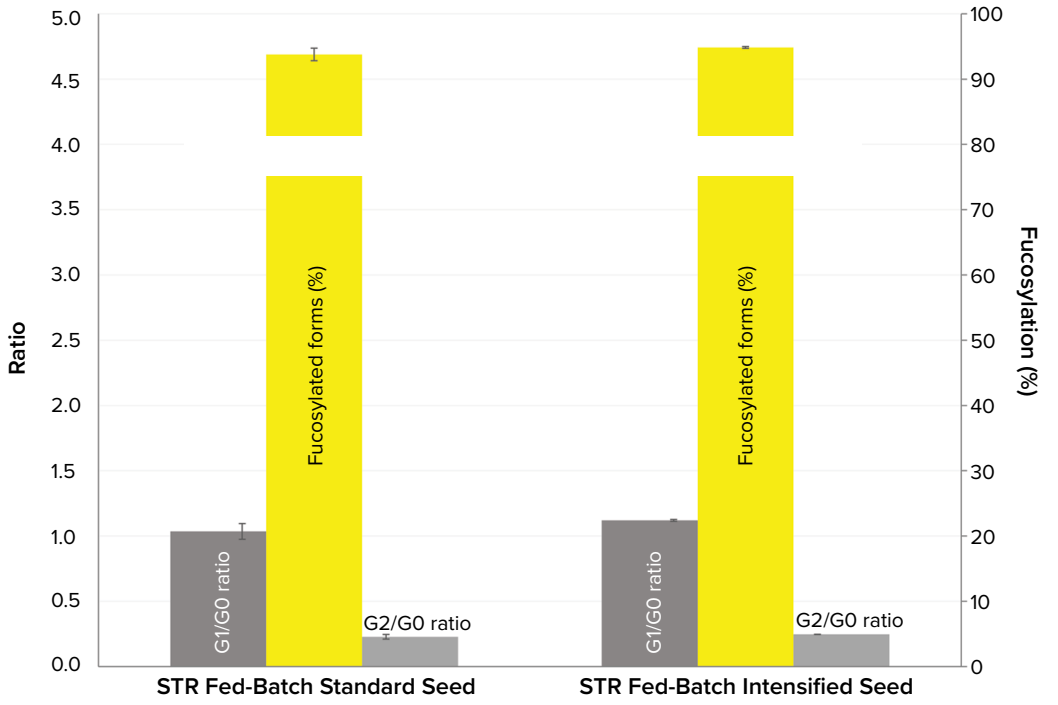
Our VCD and viability results are consistent at all scales tested up to 50 L. The reproducibility and scalability of HCD cultures using the perfusion bioreactors shows that this method can produce cells with high enough VCDs to seed 200-L pilot bioreactors directly, indicating the potential for inoculating 2,000-L production bioreactors using a suitable HCD culture, e.g., by using a RM 10 or 20.

Pilot-Scale Study: Cells in proprietary media from the RM perfusion bioreactor were used to seed a 200-L pilot-scale bioreactor using an automated PAT sensor-controlled inoculation set with a target initial cell density of 0.33 ± 0.04 × 10^6 cells/mL. That resulted in an actual starting cell density of 0.37 × 10^6 cells/mL, demonstrating that the PAT sensor can achieve inoculation precision comparable with manual seeding. In total, 0.4 L of culture from the intensified seed train was used to inoculate the pilot-scale bioreactor compared with needing over 13 L of cell culture from a standard seed train. Thus, using HCD cells to seed the pilot-scale bioreactor required over 30-fold less inoculation volume than when using cells from a standard seed train.

Fed-batch results (Figure 2) from the pilot-scale bioreactor seeded with HCD cells from the RM perfusion bioreactor and the traditional fed-batch cell culture show comparable growth characteristics over the entire process run. Both achieve a maximum VCD of 25 × 10^6 and viability of >90% in seven days. The MAb titers also were similar, with 3.5 g/L achieved using the standard seed train compared with 3.2 g/L from the intensified seed train. Those results indicate that using HCD cells to seed pilot-scale bioreactors has no adverse effect on cell growth, viability, or titer over a standard 12-day fed-batch run at 200-L scale in a single-use STR bioreactor.

Product Quality: CQAs of the MAb product from fed-batch cultures inoculated with standard and from intensified seed trains (Figure 3) showed similar trends in glycan profiles of G2/G0 and G1/G0 ratios and with 95% fucosylated forms produced. The performance

Figure 3: Comparison of MAb critical quality attributes (CQAs) expressed by Chinese hamster ovary (CHO) cells in 200-L pilot-scale fed-batch culture inoculated with cells from standard or intensified seed trains



results of the cells cultured in the pilot-scale bioreactor from the intensified seed train show that using HCD cells to seed a pilot-scale bioreactor does not affect product quality. That indicates the usefulness of this approach for a hybrid perfusion/fed-batch process scale-up.

Productivity and Quality

The proof-of-concept studies presented here show that single-use RM perfusion bioreactors can achieve HCD cell cultures (>100 × 10^6 cells/mL) with >90% viability at every scale from 1 L to 25 L working volumes. The studies also demonstrate that HCD cells (inoculated by means of automated process control) and cells from a standard fed-batch culture used to seed a 200-L STR bioreactor produce comparable cell growth, viability, titer, and MAb CQA profiles. Our results indicate that using this intensified seed-train approach can increase productivity without compromising product quality and could potentially increase the cost-effectiveness of biologics production.

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