Intensification of the fed-batch process using N-1 perfusion

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Introduction

Process intensification refers to strategies to maximize your facility utilization and increase facility output. Although this typically involves the design-in of continuous processing, fed-batch culture is still mostly used in stable protein production. Hence, strategies to intensify your fed-batch process can potentially increase productivity and reduce cost in biomanufacturing using mammalian cell culture.

Perfusion processes support high cell densities with retained exponential growth at high viability. This makes them interesting for production processes and seed train intensification. The up to 10-fold increased cell density achieved by perfusion can be used to decrease the size or the number of seed reactors resulting in reduced facility footprint and investment cost.

Using the high final density to seed the fed-batch production bioreactor at a higher cell density shifts the early growth phase in fed-batch production where titer is low, to the smaller, less costly N-1 seed culture reactor. This shortens the production duration while titers remain the same.

This strategy can remove bottlenecks in production plants and increase the volumetric productivity, which results in intensification of the fed-batch biomanufacturing process.

In this poster, we present proof of concept data to show that an N-1 perfusion process can be introduced in your seed train as a way of intensifying your fed-batch process, resulting in reduced footprint, and or increased production capacity without affecting the production process performance.

Development of an N-1 perfusion from ReadyToProcess WAVE™ to XDR-50 and APS

Methods

An N-1 perfusion process was developed at 5 L and 25 L working volume (WV) scales in a ReadyToProcess WAVE™ 25 bioreactor (Fig 1). Cells were cultured in a media mixture of HyClone™ brand ActiPro™ media and Cell Boost™ 1 and 3 (Cytiva) supplements at a perfusion rate of 40 pL/cell/day. The process was scaled to an Xcellerex™ XDR-50 bioreactor connected to an Xcellerex™ APS system. APS is a single-use perfusion skid using hollow fiber filters in TFF mode (Fig 2).

Results

- In ReadyToProcess WAVE™ 25, cells grew exponentially to densities in the range of 70 to 95 MVC/mL (Fig 3), with a total media consumption of 4.5 reactor volumes.
- In XDR-50, cells grew exponentially to a cell density of 179 MVC/mL (Fig 3) with a total media consumption of 14 reactor volumes.
- The data show excellent alignment for all three cultures and between the two types of bioreactors (Fig 3).

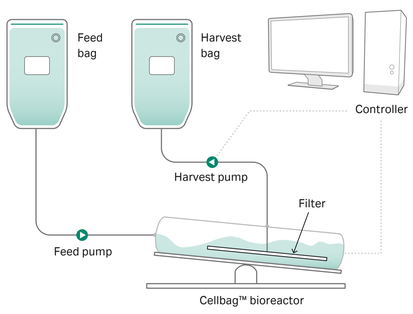


Fig 1. N-1 perfusion setup in ReadyToProcess WAVE™ 25 bioreactor.

Fig 2. N-1 perfusion setup in XDR-50 and Xcellerex™ APS.

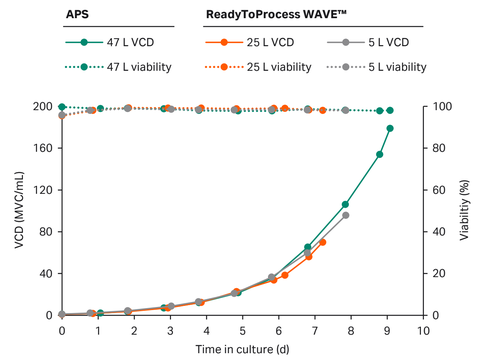


Fig 3. Growth and viability for the N-1 perfusion process in working volumes of 5 L and 25 L in ReadyToProcess WAVE™ 25, and 47 L in XDR-50 and APS.

N-1 perfusion does not affect the final process or product

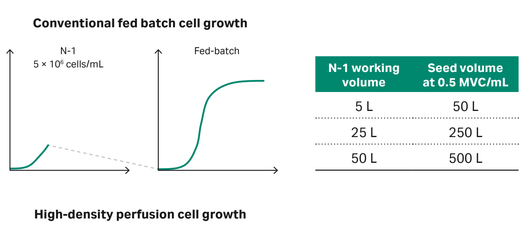
The up to 10-fold increase in cell density achieved in N-1 perfusion can be used as a way of decreasing the size or the number of seed reactors resulting in reduced facility footprint and investment cost.

Methods

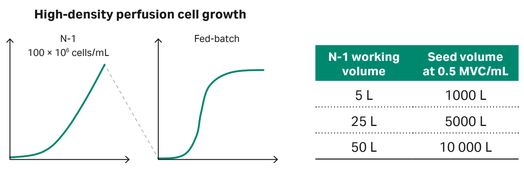
Cells perfused to 179 MVC/mL in XDR-50, (Fig 4) were seeded for fed-batch cultures in ActiPro™ medium with Cell Boost™ 7a and 7b supplements along with cells cultured in N-1 batch.

Results

The production processes align well in growth and titer as well as product quality attributes (Fig 4 and 5).



N-1 working volume	Seed volume at 0.5 MVC/mL
5 L	50 L
25 L	250 L
50 L	500 L



N-1 working volume	Seed volume at 0.5 MVC/mL
5 L	1000 L
25 L	5000 L
50 L	10 000 L

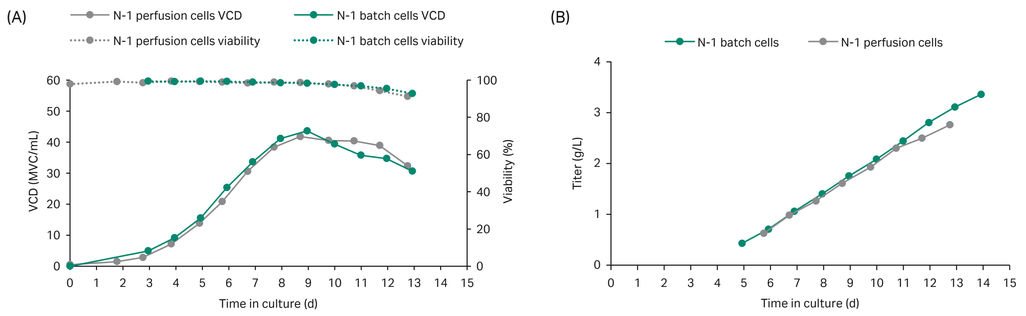


Fig 4. (A) Growth and viability data for fed-batch cultures with perfused cells and batch-cultured cells. (B) Titer data for fed-batch cultures in shake flasks seeded with perfused cells and batch-cultured cells.

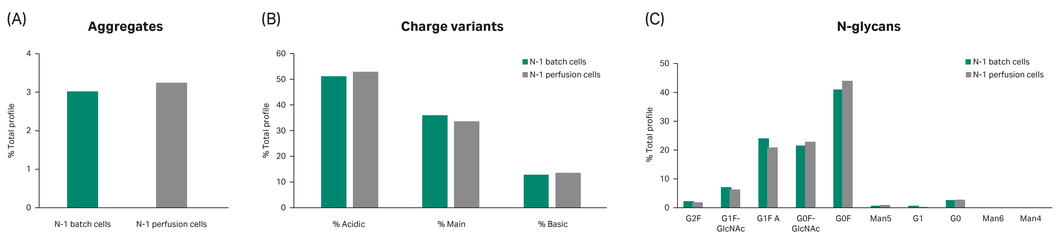


Fig 5. Product quality profiles for fed-batch cultures seeded with perfused cells and batch-cultured cells. (A) aggregates, (B) charge distribution, and (C) N-Glycan profile.

High-seed using N-1 perfusion shortens process duration

Using the high final density of an N-1 perfusion to seed the fed-batch production bioreactor at a higher cell density reduces production duration without effecting the growth or titer profile.

Methods

A conventional seed train with N-1 in batch and an XDR-500 production reactor was seeded at a cell density of 0.8×10^6 cells/mL and used as a baseline. For the intensified process, the N-1 step was performed in perfusion mode and used to seed the bioreactor at a 10-fold higher density. The production cell culture was performed in fed-batch using ActiPro™ medium and Cell Boost™ 7a and 7b supplements. The high-seed culture was supplemented with Cell Boost™ 7a Supplement and Cell Boost™ 7b before seed.

Results

- Growth curves in Figure 7 show that the growth rate after seeding is maintained with no indication of stalling.
- The high-seed growth profile is the same as for the conventional process, with comparable peak VCD and titer.
- The increased seed density shortened the production process duration by 3 days (d), Figure 7.

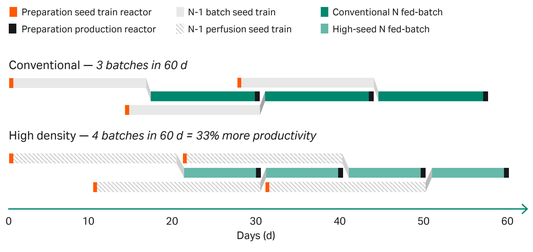


Fig 8. Scheduling a single bioreactor facility with either a conventional or a N-1 perfusion high-seed process.

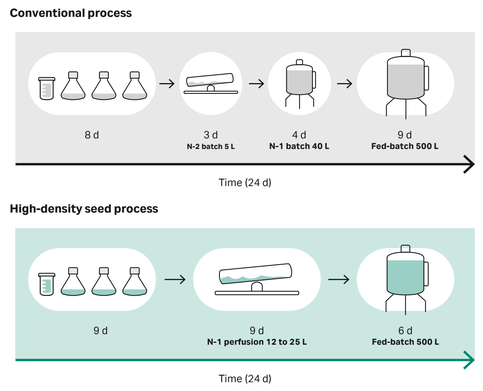


Fig 6. Overview of the conventional vs the intensified high-density seed process evaluated in the study. N-1 perfusion eliminates one seed train bioreactor and shortens the production process (6 d compared with 9 d for conventional).

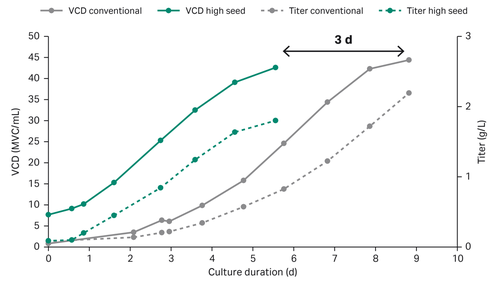


Fig 7. Growth and titer of the conventional (gray) vs high-seed perfusion process (green). The increased seed density reduced production duration by 3 d.

Figure 8 shows an example were a high seed allowed shifting 4 d of the fed-batch process to the seed train increased the number of batches from three to four in a 60-d period, representing a capacity increase of 33%.

In a multidrug facility, this can be translated to an increase from three to four drugs that can be produced in a time span of 60 d.

Conclusions

- N-1 perfusion can potentially allow very high-density seed cultures of up to 179 MVC/mL compared with only 5 MVC/mL using conventional methods
- Seed train perfusion processes are easily scaled between ReadyToProcess WAVE™ 25 and XDR bioreactors showing that it can be used as a seed train bioreactor and for process development for stirred tank bioreactor perfusion processes
- Introduction of N-1 perfusion into the seed train does not affect the growth, titer, or product quality profile of the N production process
- Process intensification of the seed train by the introduction of perfusion expands the productivity of your facility and potentially reduces costs
- The high-seed N-1 perfusion process allowed the production bioreactor run to be shortened, resulting in a 33% increased productivity compared to a conventional fed-batch process

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